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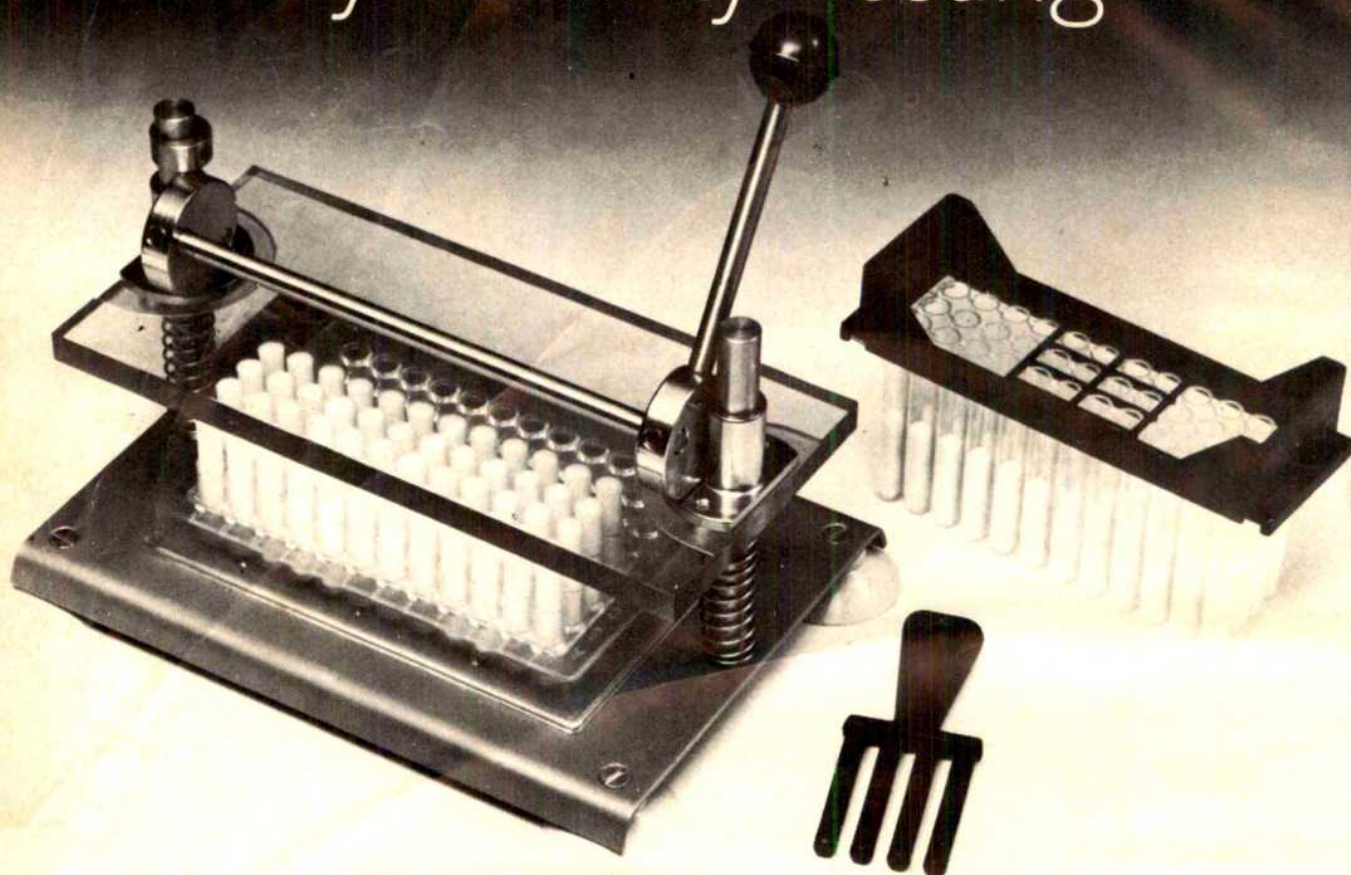
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Venus in ultraviolet light from
450,000 miles. Atmospheric tides on
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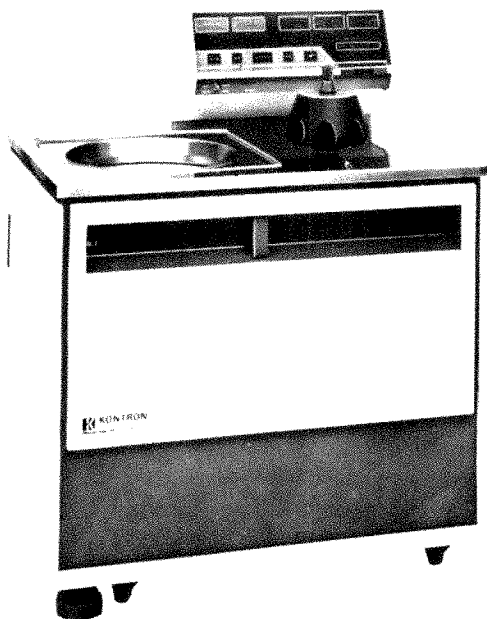
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7 September 1978

Pakistan needs indigenous medicine

CHINA'S bare-foot doctor, little-educated, trained in his art for only six months, is a constant goad to Pakistan's health-care officials. Why can't Pakistan have similar people? For the conventional doctor with his Bachelor's degree (a five-year, full-time course in medical sciences) is highly expensive and scarce. And even if available, he is not mentally prepared to leave the attractions of the city for the rural areas. So he is ill-equipped to use his medical knowledge.

About three-quarters of Pakistan's people live in the remote countryside, and successive governments have committed themselves to provide a semblance of scientific rural health cover. But there is a largely untapped option in Pakistan, similar to the barefoot doctors of China: the indigenous medicine practitioners. Called the 'hakim', if they practise Greco-Arabic medicine ('Unani Tibb' locally), and 'ved' if they practise 'Ayurvedic', the ancient Indian system, they flourish in the countryside. Could they not be the raw material for a Pakistani bare-foot medical service?

Health care in Pakistan, as in almost the whole of South Asia, is a complex mixture of elements quite different from those in the west. For instance, there are in all about 12,000 conventional doctors in the country for a population of 70 million—hardly one doctor for every 6,000 persons. In practice these provide for only one-fifth of the population. Such a low ratio (for comparison, one doctor to 641 persons in the US, with 45 categories of health personnel to assist him) creates a health care vacuum that fills itself through hakims, veds, homoeopaths and even simple charlatans.

Some 5,000 qualified hakims with diplomas from Unani Tibb colleges already contribute to rural health care; but there are also another 40,000 unqualified hakims who have no formal education nor any medical training. These hakims have learnt just by sitting beside an experienced practitioner in his clinic. Nevertheless, they are recognised and registered practitioners of medicine in Pakistan.

As far as the norms of modern medical science are concerned, even the qualified hakims coming out of the institutions after a 4-year course have a motley education. Along with Greco-Arabic system—which largely works on empirical basis—the hakims are taught several subjects of modern medicine. So the 'kulyat-e-qanoon' of Avicenna is in the curriculum alongside books on modern physiology, anatomy and so on.

Dr Mazhar H. Shah, a Fellow of the Pakistan Academy of Sciences, writes in his recently-published book *the Unity and Integration of Medicine*, that "the teaching of modern subjects [to the hakims] is by people not really qualified to teach; and there are no facilities for laboratory diagnosis and hospital treatment. The result is that Tibb colleges

produce hakims who wear the stethoscope and prescribe both indigenous as well as imported drugs without any knowledge of basic science or pharmacology. This sort of teaching and practice is neither safe for the seriously ill, nor useful to the national health system".

The cost of modern medicine to provide health care to the entire population of the country, on the other hand, appears simply prohibitive and beyond the reach of a developing country. The medical bill for health services alone in Western countries far outweighs the total of the national budget of Pakistan.

So there is a growing realisation that some sort of integration is imperative between expensive modern medicine and the inexpensive indigenous system, which is largely based on cheap local herbs. Thus the hakim and the ved could be taught the essential principles of the indigenous system but also be adequately equipped with basic principles of modern medical science; and not in half-baked fashion as at present, but in a real professional way. A village doctor emerging from such a scheme could use his discretion to use either the local herb or, if available, the relevant Western drug.

The envisaged integrated system, however, may not find smooth sailing in Pakistan due to the many vested interests. The hakim and the ved is generally opposed to integration, as at present he tries to equate himself in social status with the fully qualified western doctor. So does the homoeopath (homoeopathy is a recognised system of medicine in the country). Strangely enough, a good cross-section of western doctors is also opposed to integration as they think that it would down-grade them if systems based on empirical principles are made to dovetail with scientific methods.

Political expediency under pressure from the practitioners of indigenous systems led the Government to set up Autonomous Boards for Tibb and Homoeopathy in 1965 so as to let them "train and practise their systems independently by supplementing such modern advances as the Board may, from time to time determine." However, the government also set up (in 1974) an Indigenous Medicines Commission with Dr Salimuzzaman Siddiqui, the chemist, as its chairman to investigate the possibility of integration of the three systems. The Commission's report was never made public, but it is well-known that it had recommended integration to produce a health service structure on the lines detailed above. But a seal maybe put on the issue by the next civilian government-out of political expediency and to gain political advantage from the strong lobby of tens of thousands 'hakims'. But then that might be the end of integration and also of a practical, down-to-earth answer to rural health care in Pakistan. □

Carter juggles with fast breeder and natural gas

John Douglas discusses how President Carter has managed to enrage combatants on all sides of the fast breeder issue

PRESIDENT Carter has further entangled himself in the meshes of his energy bill — this time through an unlikely deal linking the price of natural gas with research on the fast breeder reactor.

Carter's proposals for price controls on natural gas had been bottled up in a Congressional conference committee for six months, and to free the gas bill for consideration by the full Senate, he needed one more Republican signature on the conference report. So he invited Senator James A. McClure of Idaho to the White House to discuss a compromise. Both parties deny actually making a 'deal', but McClure signed the conference report and an-

Schlesinger also committed the Administration to building the Safety Research Experiment Facility (SAREF), the Fuels and Materials Evaluation Facility (FUMEF) and the High Performance Fuel Laboratory (HPFL) — all of which the President had earlier opposed as part of his policy to shift from "early commercialisation to long-range technical development" of the breeder.

Finally came the matter of the Clinch River Breeder Reactor (CRBR), the costly and controversial demonstration project Carter has opposed since early in his election campaign. Schlesinger wrote that the project would be "discontinued" — without

own party on the issue to ignore the Republican votes Baker can influence. But more important, the compromise suggested to Baker that even if a large breeder reactor were finally authorised, the chances of building it at Clinch River, Tennessee, had been diminished.

Faced with a tough election campaign for his own Senate seat this fall, Baker reacted angrily to what he saw as a personal slight and a threat to a billion-dollar project in his home state. He announced that unless the status of the Clinch River project were cleared up he would no longer support the natural gas bill, which faces strong opposition from a small but determined group of Senators. Without his apparently vital support to cut off debate, under the rules of the Senate a minority of members might well be able to kill the bill by filibustering.

The issue of natural gas deregulation is by far the most complex and controversial section of the National Energy Act finally released by House and Senate conferees. The other three sections — dealing with energy conservation, conversion of power plants from oil to coal, and reform of utility rates — will probably pass easily, but none is likely to have the international impact of the natural gas bill, whose purpose is to lower American oil imports by substituting domestically produced gas.

The conference committee report is the latest salvo in a 39-year confrontation between producers and consumers over government control of gas prices. If enacted, the new law would eliminate the distinction between gas sold within a state and that sold between states. The two categories are now controlled differently. The bill provides that newly discovered gas in both categories would be allowed to escalate in price somewhat more rapidly than inflation until 1985, when all price controls would be removed.

The argument in favour of this loosening of control is that creation of a single gas market will make more gas available to interstate buyers, encouraging them to substitute gas for imported oil. At the same time, this argument goes, rising prices will encourage producers to step up exploration for more new gas. The Department of Energy estimates that by 1985, the equivalent of one million barrels per day of imported oil will have been replaced by domestic gas, representing a 10 percent reduction in total imports, at a \$5 billion annual savings.

Opponents of the gas bill argue that higher prices will only fuel inflation and that recent history indicates that



White House meeting: President Carter, Senator McClure, Energy Secretary Schlesinger.

nounced that he had received assurances from the White House that increased funds for a large breeder reactor no longer faced a Presidential veto. Presumably, some of these reactor funds would find their way to the Idaho National Engineering Laboratory, which has long experience in the breeder field.

Details of the understanding reached on the breeder issue were confirmed in a letter from Energy Secretary James Schlesinger, who also attended the White House meeting. The Administration, he said, would no longer oppose development of a conceptual design for an "improved demonstration project" of the plutonium breeder reactor, although the size was not specified. To fund the entire breeder program \$513 million would be appropriated for fiscal year 1979, \$504 million for FY 1980, and \$520 million for FY 1981. (The Administration had originally wanted to cut back such funding to about \$400 million for the next fiscal year, but the House had already defeated that proposal.)

specifying whether that meant "cancelled" (the earlier wording of Administration policy) or merely "deferred." Some components of the CRBR would be completed and tested, and some of the preliminary licensing would be conducted. A final decision on whether to build this or another large breeder reactor would then be made by 1982.

At first the compromise looked as if it might work. The Administration had overcome a major hurdle facing an important piece of legislation by giving in on a point on which it had already suffered a partial defeat. But in the process of hammering out this compromise, the President and his advisers committed the sort of political blunder that has too often marked the Administration's dealings with Congress: they ignored the Senate Republican leader Howard H. Baker Jr of Tennessee.

Under any circumstances, Senator Baker's cooperation would have been important to the ultimate passage of the natural gas bill, for the President faces too much opposition within his

gas companies will not rush out to drill more wells just because prices are rising. A Chase Manhattan Bank study of the question concluded that "the legislation could actually result in some weakening of the dollar as foreign exchange markets come to perceive that the long-run supply of natural gas in the U.S. is as likely to be reduced as increased." And at least two senators have announced plans to oppose the bill because of their opposition to breeder reactors, now linked to it.

The Carter-McClure compromise is also likely to broaden the debate over conduct of the entire breeder reactor programme. Work is still not complete on the Fast Flux Test Facility (FFTF) a non-power-generating breeder reactor

meant to be a forerunner of Clinch River. First authorised in 1967, FFTF was originally estimated to cost only \$87.5 million but has already passed the billion dollar mark. The CRBR has also experienced severe delays and cost overruns, and both industry and Congressional sources tell *Nature* that any further delay would probably result in a re-evaluation of the basic American breeder design.

During the debate over whether breeder reactors should be built at all, many technologists have reportedly swallowed their specific objections to the CRBR design in hopes of not exacerbating the issue. Specifically, they favour a reactor in which the radioactive molten sodium that passes through the core never leaves the re-

actor vessel and one in which steam for generating electricity is handled at a lower temperature and pressure than now called for. If the Clinch River project is deferred or cancelled in favour of producing a later—and probably larger—reactor, such arguments will probably become much more audible.

It is hard to recall a time when the US energy picture has appeared more cloudy. After he had calmed his initial anger, about the best Senator Baker could say for the recent compromise was "the water has been muddied". All that remains certain is that both the natural gas regulation and breeder reactor issues will take on new dimensions when Congress returns from its present Labour Day recess. □

Boycott of Soviet contacts is for individuals, says NAS

RECENT 'human rights' trials of scientists in the Soviet Union resulted in the immediate cancellation of several major visits by US scientists to the Soviet Union—and these in turn have provoked a biting reaction from the Soviet media—a sure sign that the threat of a severance of scientific relations was a real one. "Future historians will be amazed" wrote the *Literaturnaya Gazeta*, that such reprisals could be contemplated as a means of making the USSR give up "the principles which are sacred to it."

This reaction would appear to substantiate the case for boycott, a case most ably put by Valentin Turchin (*Nature* 273, 256–257; 1978). Not all scientists would support this view, feeling that continuation of contacts makes it possible to express one's concern with the fate of, say, Orlov and Shcharanskii, at least on the personal level, to Soviet colleagues encountered at international conferences.

Thus last month, when the International Congress of Mathematicians was held in the "human rights" city of Helsinki, right on the Soviets' doorstep (subsidised excursions to Leningrad were laid on as a side attraction), several Soviet mathematicians failed to arrive to deliver their papers. One of these people, a Dr Margulis, was scheduled to receive a medal. The ceremony was carried out in his absence, and the formal "presentation" of his work was greeted by a standing ovation, pinpointing in a telling manner the persistent Soviet practice of denying visas to scientists invited to international conferences.

Many would see the stricter insistence on the norms of scientific life—including proper representation at con-

ferences, a more fitting form of protest than an all-out or selective boycott.

Respecting the diversity of views on boycott, the US National Academy of Sciences recently put out a statement calling on world scientists to urge the release of Orlov, Shcharanskii and Sergei Kovalev, a biologist now halfway through a sentence of seven years in a labour camp. The Human Rights Committee of the Academy said:

"These scientists, along with fourteen others in other parts of the world, are of particular concern to this committee, for they represent numbers of colleagues who believe that freedom of intellectual inquiry cannot be divided into two parts—one for the natural world in which we live and one for the society which nurtures us—and who have been imprisoned for acting in accordance with their beliefs".

Nevertheless, the Academy stressed that it does not, as a body, endorse boycotts. "Each American scientist", it concludes, "contemplating a visit to the USSR (or asked to host a Soviet scientist in the US) must determine his or her own course of action."

Interestingly, this decision follows an opposite course to that advocated by a group of French physicists last July, who advocated the severance of all official scientific relations—including conferences—while maintaining personal contacts with Soviet colleagues, making the latter the "occasion of expressing our indignation".

The NAS acceptance of individual choice of protest also underlies the concluding paragraph of a moving appeal which recently reached the West from Kovalev's son, Ivan. Addressing the intending participants of the Fourteenth International Congress of

Genetics in Moscow, he outlined the history of his father's case, and gave a vivid and horrifying picture of prison conditions, he asked for the participants' support, that they would express their attitude towards the imprisonment of their colleague "by means appropriate to you". The appeal has since been circulated by campaigners for boycott, but it is noteworthy that neither Amnesty International, who distributed the letter, nor Ivan Kovalev himself, specifically ask for this.

Nevertheless, the idea of at least a selective boycott does seem to be taking hold in the USA. According to the NAS, its supporters "include scientists who pioneered in the earliest Soviet/US exchanges, seeking to build bridges of common scientific endeavour across the chasm of the cold war. They also include others who have seen themselves as steadfast in resisting the politicisation of science. People have reached their decisions in varied ways: sadness, rather than anger has been the most common emotion".

It is because the response of US scientists is so "individualistic", concludes the Academy, that continued Soviet-US scientific relations are in peril. "Scientific exchange programmes" it says "can be negotiated and organised, but individual participation cannot be commanded. There has been no institutional instruction or decision making, no rush to judgment, and no stampede to boycott. Rather there is a tide of spontaneous response running deep, and it will not be easily reversed in the absence of some judicious and humanitarian actions by Soviet authorities".

Vera Rich

Soyuz is not a trolley-bus, but Comecon flights continue

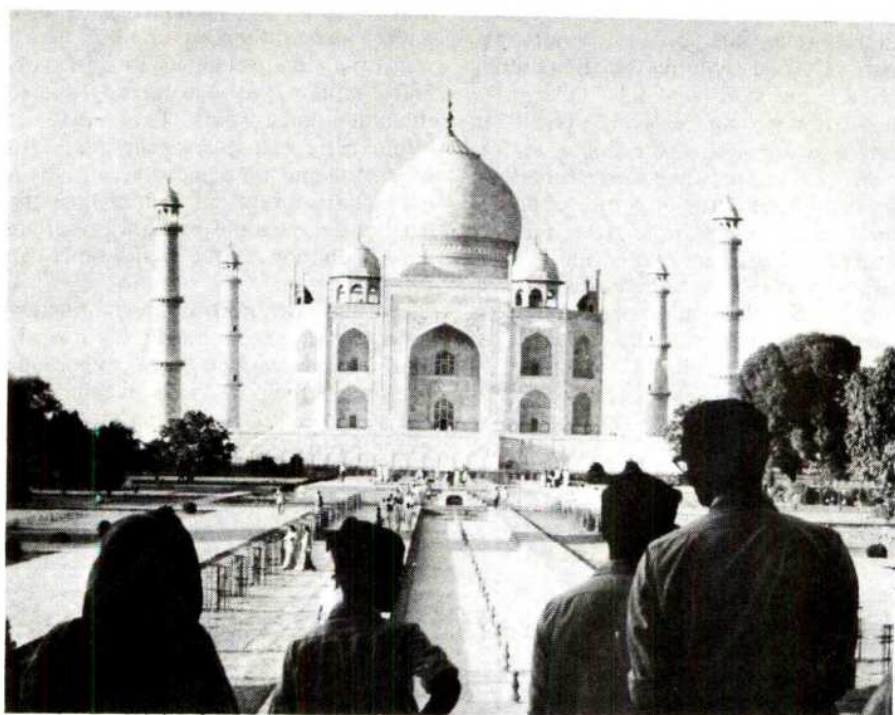
SIGMUND Jähn, the first East German cosmonaut, together with his Soviet flight-partner Valerii Bykovskii, returned to Earth last Sunday, after a week aboard the Salyut-6 orbiting station. Jähn, the third non-Soviet cosmonaut to visit Salyut, completes the first phase of "international" flights; further launches, with the participation of cosmonauts now in training from other Comecon countries are scheduled for next year.

The manned "Interkosmos" programme, in which the Soviet Union puts into orbit crew members from her Comecon allies did not at first meet with the approval of the entire Soviet scientific establishment. Academician Anatolii Aleksandrov, President of the Soviet Academy of Sciences, was reported to be against any such non-Soviet participation ("Soyuz is not a trolley-bus!"). The programme appears to have been inaugurated partly as a visible expression of fraternal solidarity, and, more practically, as a recompense for the contributions made to the Soviet space programme by the Comecon allies. (It is notable that the first three countries to be honoured by a flight were Czechoslovakia, Poland, and East Germany, whose contribution has been particularly great).

As regards Jähn's particular contribution to the joint mission, one experiment, at least, codenamed "Rech" makes use of his linguistic background. During all communication sessions, he had to repeat "Zwei hundert sechs und zwanzig", so that the parameters of his speech—tone, volume, rate, etc., could be monitored and related to his physical and psychological reaction to weightlessness. (The significance of the number 226 is not clear—nor why the experiment could not have been performed using a Russian repeating "Dvesti dvadtsat' shest'!")

Reaction to weightlessness was the underlying theme of most of the East German experiments, including tissue culture and bacteria metabolism experiments, and an investigation of the cross-linking of micro-organisms and organic polymers. This last used "a Soviet apparatus modified by GDR specialists" (essentially a complex of polythene bags).

And, as in previous "international" flights, the "Splay" furnace was used to produce semiconductor compounds—in this case lead-tellurium and bismuth-antimony compounds, the experimental capsule being renamed for the occasion "Berolina". **Vera Rich**



Taj Mahal out of danger?

THE Indian Government is considering a report from a committee of experts, headed by the chairman of the Indian Petrochemicals Corporation, Dr S. Varadarajan, on how pollution from an oil refinery being built near Agra will affect the Taj Mahal. The committee began its investigations four years ago after fears in scientific circles and in the press that sulphur dioxide waste from the refinery at Mathura 146 km south of New Delhi, would damage the white marble of the Taj Mahal and red sandstone of other historical monuments at Agra, 40 km farther southeast.

The committee's studies revealed that the sulphur dioxide content of the air in Agra is already 15 to 20 micrograms/m³ because of two power plants, numerous small industries and a railway shunting yard there. The refinery, according to the report, would only add a further 1 to 2 micrograms/m³ in the long term, although short-term peak concentrations of 65 micrograms/m³ might be expected under the worst meteorological conditions in winter.

Meanwhile the Government also engaged an Italian firm, Techneco, which specialised in the damaging effects of pollution on marble, to study the problem. The firm in its report ruled out any potential threat to the Taj from the refinery. Another study by the Indian Meteorological Department (IMD) on the expected ground level concentrations of sulphur dioxide in the Agra region because of the refinery also sought to allay the apprehensions. None of the two reports have been made public so far.

However, some experts and technical agencies have expressed doubts about the validity of the findings of Techneco and IMD which they say are based on wind data not of Agra but of Delhi. They alleged that the mathematical models, which were developed abroad, and the constants used were not appropriate for the local weather conditions. The Archaeological Survey of India, which is responsible for the maintenance and conservation of the monuments, is of the view that the emissions from the refinery would certainly discolour and wear away the white marble of the Taj.

Further critics say the Varadarajan committee has avoided the main issue: the report does not say in unequivocal terms whether the threat does or does not exist. They point out that the terms of reference did not include the possibility of shifting the refinery to another site away from Agra. (The Minister of Petroleum and Chemicals, Mr H. N. Bahuguna, had reportedly given the assurance last year that he would reconsider the moving of the refinery to Etawah, 100 km east of Agra, if the expert committee definitely said there was potential danger to the Taj.)

Nevertheless the committee recommends some control measures: that long-term emission of sulphur dioxide from the refinery should be limited to 1 to 2 micrograms/m³ and that three monitoring stations should be set up between the refinery and Agra to keep a constant check on this. The committee also puts forward plans to reduce the overall sulphur dioxide

content in Agra by closing the power plants, switching from coal-fired locomotives to diesel in the shunting yard, moving out some of the present industries and banning from the area any new ones which might add to pollution. It also wants to set up a body to monitor the air at Agra and advise industries on how to keep within acceptable pollution limits.

The Indian Oil Corporation, which has commissioned the refinery, has also taken steps to minimise pollution. Low sulphur fuel oil and gases will be used in the furnaces, and a plant will be installed for the removal and recovery of sulphur. The height of the emission stacks will be increased from 40 metres to 80 metres to ensure better dispersal, and fixed and mobile stations will monitor the concentration of sulphur

dioxide at ground level at various distances from the refinery. These measures will, according to the corporation, limit sulphur dioxide emission from the 6 million tonne refinery to 1 tonne per hour. Installing a planned flue gas desulphurisation unit could reduce this to 0.4 tonne per hour, the Corporation believes.

Corrective action is envisaged if the emissions rise above the safety level. But, critics ask, who will give directions to the refinery each time the monitoring stations give warning that the pollution has risen above the danger level? Moreover, they point out, the recommendations are not mandatory. What is the guarantee that the measures suggested by the expert committee would be fully implemented? And even if all the safeguards are taken, a certain amount of

residual pollution will cause damage to the monuments over a long period.

The latest among the public figures to come out openly against the refinery is the noted ornithologist Dr Salim Ali. In a public statement he called for the shifting of the refinery as "there is a controversy among experts about its effects on the Taj". Dr Ali also spoke about the threat to the famous Bharatpur bird sanctuary near Agra because of refinery gases.

While the Government is yet to take a decision on the Varadarajan Committee report, more than 80 million rupees is reported to have already been spent on the refinery project. There is growing apprehension among critics that it may soon be too late to turn back. □

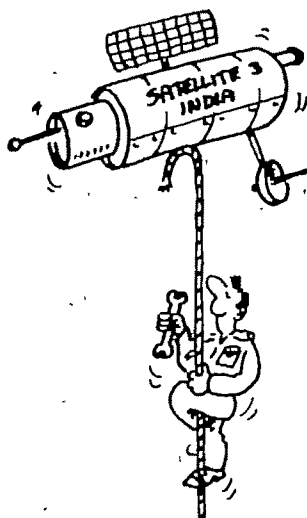
India plans to invest in tethered balloon technology

WITH the successful ascent of a 270-cubic metre tethered balloon at the Space Applications Centre (SAC), Ahmedabad, on 19 May this year, India has joined the select band of countries like the United States, Britain and France which have developed tethered balloon technology.

According to the Electronics Commission's Information, Planning and Analysis Group, India has all the expertise and indigenous materials to develop this technology on a large scale. A panel on tethered balloon technology, set up by the commission to work out the best plan given available resources and the work in progress in the country, has recommended developing balloons of all sizes—small, medium and big. The panel has also stressed the need for making possible users aware of the potential of balloons.

Research in this technology began in India when tethered balloons were seen as a substitute for the ATS-6 satellite for the continuation of the SITE programme. On the initiative of the Electronics Commission, research was taken up at SAC in April 1974. Apart from the many government and private institutes that helped in designing and testing the balloons, collaboration was sought with the Tata Institute of Fundamental Research, where work was already in progress on plastic balloons.

Two years ago, Tata launched a similar balloon to the present one from its National Balloon Facility at Hyderabad. The present balloon is of medium size, has a payload of 50 kg and can float to an altitude of 1.5 km. Except for two fireproof motors in its pressure regulation system, its components are all Indian made. It carried telemetry and telecommand equipment and batteries.



There is no doubt that tethered balloons are the poor nations' substitute for satellites. Once their technology is developed, they can be used for TV and FM broadcasting, remote sensing, ecology, oceanography, meteorology, coastline management and pollution monitoring. Because they are tethered, height can easily be varied for the study of atmospheric phenomena close to earth. A study carried out by IPAG suggests using balloons in offshore oil exploration as a temporary communication link with offshore platforms.

For communication and TV relays over hilly areas, where building earth-

bound installations is difficult and expensive, tethered balloons can also be used. The panel has said that two balloons at an altitude of 30,000 metres, one at Shillong and another at Dibrugarh, could be easily and cheaply used for a service covering most of the north-eastern hills of India.

The panel also advocates the immediate development of low technology tethered balloons—of low payload and short flight duration. For instance, a tethered balloon with a 10 kg payload, parking at an altitude of 1 km for 24 hours, could be useful to the Meteorological Department and the Institute of Oceanography. Such balloons could also be of use in the MONEX programme scheduled for the 1979 monsoon in the country. The panel has also asked for a study of hazards to the balloons, such as lightning and ultraviolet rays.

Once the technology is fully developed, it is recommended that a national agency should take over to tailor the balloons to the needs of users. How cost effective a tethered balloon can be will be apparent from one example. For a TV broadcast operation for one per cent coverage of India, the total capital investment is 40 million rupees with an annual recurring cost 4.2 million rupees. The same costs for a tethered balloon would be 6.5 million and 1.7 million rupees.

Dilip M. Salwi



Correction

In 'India prepares to launch Earth resources satellite' (10 August, page 526) it was incorrectly stated that the SEO satellite will be launched from the SHAR range in India. The satellite will be launched in the Soviet Union.

India on stand-by for a plague of locusts

INDIA is standing by for a locust invasion on its borders with Pakistan. Five wireless-linked locust control centres have been established in the border states, and chemicals, men and machines rushed in as locust activity increases. An agreement has been signed between the two countries for a joint strategy to combat the menace. Field officers on either side of the border are meeting regularly to exchange information on locust swarms, egg-laying and emergence of hoppers.

If the locust threat materialises, it will be the second invasion this year. It was in mid-June that the first swarmlets of locusts invaded India. A rare change in wind pattern over the Arabian Sea brought them to Banaskantha and adjoining districts of Gujarat from their original breeding grounds in Africa. The Plant Protection Organisation of the Agriculture

Ministry swung into action and neighbouring states alerted. Benzene hexachloride dust, dusting machines and handpump sprayers were rushed in.

Swarmlets swept in across the India-Pakistan border, and spread to Rajasthan, Haryana and Punjab. They came in small and thin waves. A swarmlet was hardly larger than 3 sq km in area—though a swarm as large as 27 sq km was also spotted in Rajasthan.

India's last locust plague, 16 years ago, destroyed half a billion rupees of standing crop. Except for spoiling leaves and some standing cotton crop in Haryana, the recent invasion did almost no damage—thanks to timely action by the Agricultural Departments of Punjab and Haryana.

Control rooms were set up and anti-locust squads formed. Villagers were trained to report any sign of locust activity. Areas where the locust swarms

had laid eggs were ploughed or sprayed with chemicals. The conventional methods of flame-throwing and digging trenches were also used in some villages, and eventually, all the swarmlets were either killed or paralysed, except one which managed to fly back to Pakistan across the Sutlej river.

The current locust concentration is in the largely desert Saurashtra-Kutch region of Gujarat. At this time of the year, heavy rains turn it into a marshland and a breeding and egg-laying ground for the locusts. Locust swarms have also laid eggs in a large number of villages in Rajasthan.

A total of 100 vehicles, five planes, two helicopters, 100 power dusters and 5,000 hand dusters have already been rushed to these border areas. Border Security forces and the Air Force are also on hand if needed.

Dilip M. Salwi

Vietnam still looking for foreign aid

VIETNAM'S appeal for more development aid seems to be having a mixed reception. Many countries and international agencies have signed technical and scientific aid agreements with the Vietnamese, but some now have reservations about starting new programmes. The United States refuses to consider any commitment to Vietnam until diplomatic relations are restored.

International organisations provide a substantial proportion of Vietnam's current aid revenue, and a number have commitments within the country. The World Food Programme, for example, has provided US \$89 million since April 1975 for emergency assistance, irrigation work, resettlement programmes in the 'new economic zones' and afforestation programmes. The United Nations Children's Fund is giving \$73 million from 1976–1979 to provide basic health care and primary education and to improve drinking water supplies. In addition the U.N. Family Planning Association (UNFPA) is already operating family planning programmes and will manufacture contraceptives; and the World Health Organisation (WHO) is, to take one example of its activities, training health care personnel.

Financial institutions such as the World Bank and the Asian Development Bank are also to make funds available for projects to improve irrigation, rehabilitate light industry, explore oil resources, and develop telecommunications.

UNESCO and the International Atomic Energy Association (IAEA)

are two of the more scientifically-orientated agencies with an interest in Vietnam. UNESCO is helping to equip the Nha Trang Centre of Oceanography and to set up the Scientific Information and Documentation Centre. (Because of its concern in the field of the restoration of historical monuments, it is also being asked for assistance in restoring the former imperial capital of Hue.) The IAEA feels it could help with the application of isotope and radiation techniques to agriculture and ground water surveys—both given top priority in the country's development programme.

Vietnam is no longer at the top of the UN list of those in need of development assistance—that position is now taken by Angola and Mozambique. But interest in Vietnam has not waned. The report back in March of the Special Commission on Assistance to Vietnam was very well attended. The Soviet Union, China, and the nations of Eastern Europe have long supplied aid to Vietnam and more recently Sweden, Norway and Holland have also established commitments in the country. They are maintaining a keen interest in the redevelopment of the country and are involved in several specific projects.

Vietnam has given science and technology high priority, but most aid agreements favour technology. Those concerned more with science, and involving Western nations, are limited to India, France, West Germany, Canada, Switzerland and the Philippines. These agreements are mainly to provide

advanced training for Vietnamese students.

Lack of technical equipment presents great problems for scientists in Vietnam. This means that research targets are not always met and—for biologists in particular—research programmes are sometimes postponed. The United Nations mission to Vietnam highlighted the needs of Vietnam's scientists, and the UN officials involved with the mission do not hide their disappointment at the apparent disinterest shown by countries supplying scientific aid. They admit, however, that they are not privy to the terms of every bilateral aid agreement and that some countries may be assisting by supplying scientists with vital equipment.

Britain, however, has no immediate plans to supply scientific aid. It is concerned with human rights in Vietnam. The Ministry of Overseas Development (ODM) and the Foreign and Commonwealth Office say that aid already committed to Vietnam will not be affected, but that no new agreements will be made until the government is reassured that human rights are not being violated.

But Britain is not alone. Sweden and Holland have expressed similar concern. They are worried about press reports of refugees leaving Vietnam; of the measures taken to restrict private enterprise in the south of the country; and of the means used to persuade people to move into the new economic zones. The British Government has also shown its concern about human rights in other countries and has

IN Britain today only about 400,000 people work full time on the land. Of these 160,000 are hired workers, 200,000 are farmers (tenants, land-owners and managers) and the rest are members of the farmers' families. Thus Britain, with a total population of 56 millions, has a smaller proportion of its citizens engaged in agriculture than has any other country, be it industrialised or what is now called, optimistically or euphemistically, "developing".

Yet notwithstanding this tiny labour force, food production in Britain is greater than ever before. Were we content with the diet enjoyed by all but the greediest and most prosperous individuals a hundred years ago, we should already be more than self supporting in all foods except things like tea and coffee which do not flourish in our temperate climate. We could survive a seige better than in the last war, when we had a smaller population to feed and nearly three times as many people working on our farms.

Farmers are, however, worried, because the small number of workers means that the industry has little political power. There is probably no single parliamentary constituency in even the most rural part of Britain where the votes of farmers, farm workers and their families would substantially affect the chances of election or rejection of a Member of Parliament.

Thus my own constituency of Huntingdonshire is perhaps the one which is most dependent on agriculture. At the last election the sitting member, Sir David Renton (Conservative) had a majority of nearly ten thousand votes. In this area there are 1,391 farmers, smallholders and market gardeners, and about the same number of employed farm workers. A few of these are below voting age, quite a number are single, and the total strength of the voting force is probably under five thousand. We do not know exactly how these

Farming lobby



KENNETH MELLANBY

people voted in the past, or how they will vote in the future. Large scale farmers are mostly traditionally Conservative, the workers trade union is to the left of the Labour Party, many smallholders are Liberal in their sympathies, so the farming vote is likely to be split. But even if 100% turned out for one candidate, the total would be less than half the present member's majority.

It is partly because their political power is so reduced that British farmers are making greater efforts to develop public interest and sympathy towards their problems. Many thousands of the general public attended the recent Royal Agricultural Show, where various entertainments were provided as well as displays of traditional farming activities. Up and down the country smaller county and district shows set out to cater for the non-farming public, giving them an enjoyable day out and a painless education at the same time. The town dweller at least learns that milk does not originate in bottles and tins, and that their daily bread comes from an arable crop which has its own pest and disease problems.

A recent development has been the organisation of "farm trails". On a Sunday afternoon in late June I went

along to a large arable farm in the fens near to my home. A conducted tour started off every hour. I joined about 150 miscellaneous men, women and children, from town and country. We mounted trailers to be drawn by enormous tractors around the fifteen hundred acre farm. The arrangements went smoothly, though the farm roads were bumpy, and one tractor (a.s., the only British made model) broke down and its load had to crowd onto the four other trailers. We covered several miles on this totally arable farm. We saw fields of wheat, sugar beet, potatoes and green beans. At strategic points we stopped to be addressed by our guide, who explained what each crop was, and the main problems it presented. We were told why they kept no farm animals. We saw immense modern machines, for instance one to harvest beans (for freezing) which cost over £50,000 and was only used for about ten days in each year.

The crops, though not yet ready for harvest, were superb. It was obvious that yields would be greatly above the national average. Those who criticise large farms for being unproductive, in yield per acre, should have been there. Hardly a weed remained in the crops, though odd corners of fields were left uncultivated or planted with native trees and shrubs to encourage wildlife. Even a few hedges remained. This may be the "agribusiness" which many of my conservationist friends so criticise, but we could see how good farmers maintain and improve the fertility of their soil, and even care for the countryside and for the conservation of wildlife. In the depression in the nineteen thirties this farm was derelict, no one was prepared to work it even at the lowest rent. It is now producing enough food to feed all the population of a small town, and its yields continue to grow year after year. The demonstration was evidently an excellent exercise in public relations.

curtailed aid programmes to Uganda and Bolivia.

On the grounds of development alone, Vietnam is regarded by the US as well as Britain as being entitled to substantial aid. But there is little chance of the US supplying official aid in the near future. 1978 is an election year, and officials say that aid for Vietnam is too controversial an issue at present.

Vietnam and the US have not established diplomatic relations; both governments insist on certain preconditions before normal relations can be

restored. Senate officials have been assured, however, that unofficial links between scientists and institutions will not be opposed. Some US scientists are not convinced of the interest of Vietnamese scientists in such exchanges; offers of funding for study in the US have not aroused much response, and several scientists who visited Vietnam in the hope of studying the effects of herbicide spraying found the Vietnamese unhelpful. Such an attitude, however, is not without its political overtones (see *Nature* 271, 597; 1978). For this reason, some

scientists consider that it would be rash to prejudge the Vietnamese on their sensitivity over this issue.

US and UK attitudes to aid to Vietnam are unlikely to have disastrous consequences for Vietnam's current development programme. There could be problems, however, if more countries adopt similar policies. In this case Vietnam's scientists, desperately struggling to overcome the ravages of war on their research programmes would be some of the first to suffer. It is political decisions which will determine the outcome. **Alastair Hay**

correspondence

Nitrates and nitrites cannot be eliminated

SIR,—The report that nitrite has induced cancer in animals is not surprising as it has long been known to be mutagenic. The benefits from the use of nitrite as a food additive may outweigh the hazards that it presents as a mutagen and carcinogen. The amounts of nitrite in food either 'naturally' or as additives should obviously be kept to the minimum effective levels. Small amounts of nitrites and nitrates are formed by the electric discharge of lighting and it is difficult to avoid this contaminating drinking water.

Nitrate is, however, formed by the decomposition of organic matter and indeed the nitrate used for gunpowder was made in this way until the deposits of Chilean saltpetre became available in the nineteenth century. In conditions of poor sanitation the nitrate formed by "decomposition of organic matter" including faecal residues will be a pollutant. In dry regions this is likely to be present in air as a fine powder and in moist areas as a water contaminant. Improvement in hygiene could therefore remove or reduce a carcinogenic hazard. Nitrite is, however, formed in the intestine by bacterial action (Tannenbaum, S. R., Fett, D., Young, V. R., Land, P. D. & Bruce, W. R. *Science* **200**, 1487; 1978) and it is difficult to see how this could be prevented.

Nitrates are converted to nitrites in the body. Nitrites could be carcinogenic directly by reacting with the purine residues of nucleic acid. They can also act by reaction with secondary or tertiary amines to form nitrosamines which may be metabolised by tissue enzyme to alkylating agents.

The new findings increase the correlation between mutagenicity and carcinogenicity and indicate that exposure to nitrates and nitrites should be reduced although they cannot be completely removed.

Yours faithfully,

E. BOYLAND

*The London School of Hygiene
and Tropical Medicine*

Foxing fungus

SIR,—The interesting letter from Maynell and Newsam (3 August, page 466), confirming the suggestion that foxed paper has suffered from fungal attack, raises the question "Why was this form of deterioration called *foxing*?" The dictionary explanation is that the word refers to the brownish colour. If that were all, rusting would have been a more suitable word. I have often wondered whether it is merely a coincidence that several harmful effects, now known to be caused by fungal growth, are verbally related to the fox. For example: fox-evil, now called alopecia from the Greek for a fox; wine fermented by a contaminated yeast culture; and beer that has 'gone off'. Robert Graves argued somewhere

that fox in the Bible is often a mistranslation of *mushroom*. For example: "Take us the foxes, the little foxes, that spoil the vines" (*Song of Solomon* 2 15). He explained the extraordinary story (*Judges* 15 4,5) of Samson tying firebrands to the tails of pairs of foxes, and sending them off to burn the Philistine's crops, thus: "he inflamed his soldiers by feeding them on mushrooms (? *Amanita muscaria*) and sent them in pairs to destroy the crops".

I wonder whether there could have been a similar confusion in other languages. The metaphorical use of fox to describe colour or cunning is understandable. The uses of the word that are discussed here predate recognition of the fungal category. Nevertheless, they all have some connection with observable growth. We get fox from a Teuton rather than a Latin root. Could there have been a confusion between the German *wuchs* and *fuchs*. In English, the f becomes a v in vixen. It would be interesting to know whether any scholarly person has thought about the matter. It's not my subject.

Yours faithfully,

N. W. PIRIE

Harpenden, UK

Compulsory boycott

SIR,—Unlike many other people, I never intended to boycott the Fourteenth International Congress of Genetics of Moscow, August 1978. I was very anxious to meet our Russian colleagues. Five communications from members of my department were accepted by the conference organising committee. Two of their authors had even been invited to act as co-chairman for some of the congress sessions.

But the Soviet authorities refused to grant me an entry visa, without giving any motive. To show their solidarity, my collaborators decided to cancel their participation in the congress.

In answer to my protestations made by telegram, I received (on 18 and 19 August) two calls from the organising committee, assuring me that there had been a technical mistake which would be rectified promptly. I was asked to wait for further information. I was still waiting, when the congress was almost at an end.

Who has boycotted the congress? Not I! But will my Russian friends hear of it?

Yours faithfully,

ANTOINE ELENS

Namur, Belgium

Making mincemeat of our common language

SIR,—An American visiting Britain, and wishing to cook a hamburger (whatever its load of carcinogens) is in for a "gastronomic surprise if he acts on the information given by my friend Tom Jukes

(27 July, page 307). If, in an attempt to purchase ground beef he asks for mincemeat, he will receive "a mixture of currants, raisins, sugar, suet etc." (see the Oxford Dictionary), the stuff we British use at Christmas to make the traditional mince pies. If he is lucky, there may even be some brandy in the mincemeat; a hundred years ago our more virile ancestors included steak, but fruit and sweet substances always predominated.

The English synonym for ground beef is simply mince. The shopkeeper would understand what was needed if asked for minced beef.

Yours faithfully,

KENNETH MELLANBY

*Monks Wood Experimental Station,
Abbots Ripton, Huntingdon.*

Marsh mallow grows in Britain

SIR,—Rosemary Angel, in her review (4 May page 79) of *Major Medicinal Plants: Botany, Culture, and Uses*, takes the author, Julia F. Morton, to task for saying that marsh mallow is grown in gardens in Great Britain. "No, I am afraid it is not," she says. Without wishing to be picayune, I feel I should say that it is. I have grown marsh mallow in two successive gardens, and it is one of the most beautiful plants I know. It is growing in my garden now. And I am well aware of the difference between common mallow, musk mallow, marsh mallow, and the others.

Yours faithfully,

ROBERT K. G. TEMPLE

Bridgwater, Somerset, UK

Sex and cancer

SIR,—In his review of A. C. Braun's book, *The Story of Cancer: On its Nature, Causes and Control*, Richard Peto says, "... many, and perhaps most, cancers are caused by certain sexual habits, smoking habits, and gross aspects of diet rather than by contaminants."

For God's sake, Peto, which habits? Are thousands, perhaps millions of human beings to perish through your reticence (or bashfulness)?

And if—as can hardly be doubted—the Regius Professor is correct, can he tell us which sexual habits of plants cause their cancers? Or which smoking habits?

Yours in fear and trembling,

RALPH CHERNOFF

Los Angeles, CA, USA

news and views

Thin films: present and future

from Robert M. Hill

TWENTY-FIVE years ago, as part of an undergraduate course, I was introduced to the world of thin films. In an evacuated bell-jar a sample of aluminium wire was heated up by a tungsten filament, the aluminium melted then vaporised and condensed on the cold surfaces of the bell-jar and on a microscope slide mounted in a holder. I think the rest of the experiment was to measure the optical transmittance and reflectance of the semi-transparent metal film. The feature that caught my imagination was the extreme thinness of the metallic deposit, somewhat less than 30 atom layers. For the first time atomic dimensions were realisable, structures could be seen to grow and one could visualise the packing of atom layer on atom layer and investigate the physical effects of size down to angstrom dimensions.

The same period of 25 years has seen an amazing growth in the technology of thin films. New methods of deposition, or rather more correctly, improved techniques based on the development of old ideas have become available, a new range of instruments for investigating the structure and properties of films have been developed and there have been major steps in our understanding of the delicate processes of nucleation and growth, and of the effects of structure on the physical properties of films. A major step in the recognition of thin film science was the establishment, in 1966, of a journal, *Thin Solid Films*, specifically devoted to the publication of scientific papers on thin films and their properties. The journal has now brought out its 50th volume (gone are the days when volumes and years kept pace together) and has taken the opportunity of asking the editorial board and contributors to present their personal views of the past and of the future. Scientists, as we all know, are notoriously reticent

about forecasting the long term future, unless one manages to be party to a group relaxing together after a long day listening to interminable papers at a conference or summer school, and there is little crystal ball gazing in this anniversary issue but there is an excellent set of individual views of the past, and particularly the present, state of the field.

The methods of preparation of thin films are many. In this volume alone are descriptions of deposition by vacuum evaporation, chemical vapour deposition, ion beam methods, gaseous adsorption, photodeposition, plasma polymerisation, epitaxial overgrowths and the stacking of monomolecular layers. In principle it seems that any material, element, alloy or compound, can be grown in the form of a thin film. Indeed many alloys can be deposited in metastable forms that are not known in the bulk. The most perfect layers are those grown by epitaxy on single crystal substrates, a technique widely used in the preparation of electronic devices where the interface between film and substrate, or between two different films, of good crystal structure has to be sharp and well defined. Compound formation can be aided by vapour deposition in which chemical reactions are allowed to take place either in the vapour phase or at the growing interface. Much of the recent work on the electrical properties of amorphous semiconductors was carried out on thin film specimens in which the normal crystallisation processes were inhibited by holding the substrate at such a low temperature that the initial surface mobility of the vapour atoms was inhibited and hence the required non-crystalline form obtained.

The wide range of uses of thin films is also clearly shown. The electronics industry is an obvious candidate in these days of planar technology but the dielectric properties of oxides and the low temperature coefficient of electrical resistance of both alloys and mixed metal/dielectric structures have been

made use of as capacitors and resistors for many years. A new area of growth is that of integrated optic circuits in which optical signals are processed as they pass along thin surface films of dielectrics, equivalent to flat optical fibres. This is, in some ways, an extension of the already long established use of thin films as blooming coatings on optical lenses and in multi-layer interference filters. Here too there have been many recent advances and new problems uncovered particularly by the use of high energy density optical beams from laser sources. Twenty-five years ago the coating of the Mount Palomar reflector with aluminium was a major task, how many mirrors, prisms and lenses have received a thin film coating since then?

In many areas the future prospect for thin films looks good. Taking only two examples the journal reports significant advances in the thin-film-transistor and in film based solar power cells. By itself the t-f-t cannot compete with silicon technology but for a flat plate television display there is a need for a simple, cheap, mass producible, amplifying device at the end of each (x,y) coordinate. Here thin film technology can play its part and the problems that have been encountered in developing a reliable device are being overcome. The present high cost of solar cells is primarily due to the single crystal silicon used as the starting material. In order to bring the cost down to be comparable with that of conventional power supplies by 1986, as has been proposed, non-single crystal material will have to be used, and in the form of thin layers. The journal reports that thin film technologies should achieve the target on time.

It will be interesting to see what is reported in the 100th volume of *Thin Solid Films*. Perhaps by that time scientists will not be so reticent about the future. For the present the Editors are to be congratulated in giving their authors the chance to look round the field and giving us an end of term report.

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Antibodies to probe development

from John K. Heath

THE favoured method in recent years for investigating the cell surface of the early mammalian embryo has been the use of antisera raised against specific embryonic tissue, or teratocarcinoma cell lines.

The best characterised of these is probably the antiserum raised against the F9 teratocarcinoma cell line (reviewed by Jacob *Immun. Rev.* **33**, 3; 1977). This antiserum reacts with a fairly restricted set of tissues; the pre-implantation embryo, the germ line and other teratocarcinoma cell lines. The fact that a particular antiserum reacts with a particular set of tissues is not in itself very informative about the role of the cell surface in the development of the early embryo. These reagents do however provide basic information on a population of cells at the molecular level, and often show that cells which are morphologically homogeneous are unexpectedly heterogeneous in their expression of cell surface antigens. F9 antiserum, for example, reacts with nearly all F9 embryonal carcinoma cells (EC cells) but with many fewer, morphologically identical PCC4 EC cells (Seisner *et al. Devl Biol.* **61**, 20; 1977). This and other observations are potentially important because 'classical' experimental embryology is usually based on histological analysis, and has generally classified cells into morphologically discrete types. This has led to a tendency to view the cells in a developing organism as moving through rigidly defined discrete stages. The use of antibodies not only allows one to extract more information on the effects of classical embryological manipulations, but poses questions which would never have arisen if cell morphology alone were used as a guide.

Conventional antisera can only be raised against those antigenic determinants which elicit sufficient response to produce detectable levels of antibody in the serum. Many potentially interesting molecules therefore escape detection. Conventional antisera are also often a complex mixture of antibodies directed against different determinants.

The recent development of 'hybridomas'—cell lines producing specific monoclonal antibodies—provides a way around these difficulties. If spleen cells from an immunised animal are fused with a myeloma cell line some of the resulting hybrid clones secrete antibody directed against the original immunogen (Kohler & Milstein *Nature* **256**,

295; 1975). Each individual clone is descended from a single antibody-producing cell in the original animal and so makes antibody directed against a single antigenic determinant. These monoclonal antibodies promise to revolutionise the study of the early mammalian embryo, amongst many other systems. They dispense with many of the technical drawbacks of conventional antisera, and allow one to find molecules hitherto undetectable on the cell surface. The first papers applying monoclonal antibodies to the study of the early mammalian embryo are now available (Stern *et al. Cell* **14**, 775; 1978; Willison & Stern *Cell* **14**, 785; 1978) and many more will doubtless follow. The results of these studies again show up unexpected differences in the expression of cell surface molecules, which might not have been predicted by classical experimentation. In making hybrids between spleen cells and myelomas one is effectively sampling the whole antibody response to a complex immunogen, and it is therefore possible to make antisera directed against very minor determinants in a population of cells. Indeed, the original immunogen used by Stern *et al.* was a preparation of lymphocytes, partially enriched for T cells. One of the clones resulting from the fusion made a product which reacted with a minor subpopulation of lymphocytes (about 2%) but reacted with EC cells to a much greater extent. This illustrates the value of screening the products of hybrids against a panel of cell types other than the original immunogen. Further investigation showed that the antibody produced by this particular clone reacted with sheep red blood cells, brain and kidney as well as with some somatic component of the testes. The species and tissue distribution suggested that this antigen was similar to Forssman antigen, and the case for this was strengthened by the observation that the antigenic determinant was carried on a glycolipid molecule.

When attention was turned to the preimplantation embryo however, the distribution of this single determinant turned out to be most unusual. The determinant first appeared on the late morula stage (16–32 cells), but could not be found on all embryos. The amount of staining increased as the trophectoderm formed, but again not all trophectodermal cells reacted with the antiserum, and there was no obvious pattern of reactivity common to all embryos. In some cases it appeared as though only one or two cells in the trophectoderm carried the determinant. If the embryo was allowed to grow out

in culture (mimicking the process of implantation) the determinant disappeared from the trophectoderm completely.

The strange distribution of this determinant does not fit in with any preconceived notions of trophectodermal formation or differentiation. It is possible that the expression of this determinant is related to the cell cycle, or that the molecule is perhaps somehow masked in the cell membrane. In any case these findings call for a number of experiments which would not even have been conceived or made possible without antibodies.

Many embryologists believe that the cell surface has an important role in development. There is, unfortunately, very little direct evidence to comfort them. Perhaps monoclonal antibody technology will not only stimulate experiments to reassure the faithful, but help to challenge a number of long held beliefs about developmental processes in general. □

History of biology at the Naples Zoological Station

from Robert Olby

Few biologists can be unaware of the important contribution which the Stazione Zoologica di Napoli has made over the years since the first visiting scientist came to work there in 1873. As Theodor Boveri declared—"How many prosperous investigations, how much joy of discovery, has this house known!" Established by the wealthy German zoologist and student of the origin of the vertebrates, Anton Dohrn, the station was intended to foster internationally the study of marine organisms. As an enthusiast for the Darwinian theory, Dohrn believed that comparative studies of the development of higher and lower marine organisms would provide the key to the phylogeny of the vertebrates. In fact the Station has been the site for researches of very wide significance in a variety of fields. They include Boveri's classic researches into nucleocytoplasmic relations and the individuality of the chromosomes in the sea urchin egg, and Otto Warburg's measurements of the respiration rate of eggs before and after fertilisation, a landmark in the introduction of physiology into development biology. Others who carried out researches at the Station were

* Held at the Naples Zoological Station Annexe on the island of Ischia from July 1–15.

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August Weismann, von Uexküll, T. H. Morgan, E. B. Wilson, Albert Szent Györgyi, Jean Brachet, Marcel Florkin and J. Z. Young.

Anton Dohrn had financed the Station from four sources—receipts from visitors to the Aquarium (then as now housed in the Station) contributions from institutes and governments who rented 'working places' for visiting members from their institutes, proceeds from the sale of preserved marine animals, and contributions from patrons. This private status of the Station came to an end as a result of reorganisation under a new Administrative Council formed in 1975. Two directors have served under this Council, Alessandro Barlaam and the present director, Alberto Monroy.

Both directors were aware of the importance of the archival material at the Station. Several years ago the archivist Christiane Groeben sorted and catalogued the scientific correspondence, photographs, and other documents with the aid of funds from the Dohrn family Foundation and the Deutsche Forschungsgemeinschaft. In 1974 an exhibition of this material was held at the Station; copies of the excellent catalogue are still available.

As the existence of this remarkable collection of some 15,000 letters is becoming more generally known Groeben's task is growing. The historical work of the Station is being expanded with the formation of a Research Group consisting of Bernardino Fantini of the Institute of Genetics in Rome, Christiane Groeben and the Station's librarian Walter Groeben.

The aim of the group is to promote the history of embryology, cell biology, biochemistry, neurophysiology and taxonomy, with special attention to the social and cultural role of research institutes. In addition to the organisation of the scientific correspondence at the Station, scientists who have had connections with the Station will be interviewed. It is planned to start with J. Brachet, A. Monroy, G. Montalenti, M. Aloisi and J. Z. Young.

At the first of a proposed series of biennial summer schools, this year on biochemical and molecular embryology*, Jean Brachet gave a spirited account of his involvement in chemical embryology. The ways in which the subject of the distribution of nucleic acids in the cell was affected by the lack of congruence between the disciplines of biochemistry and histochemistry, and the impact this had on his scientific career came over clearly.

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Petrified cotton?

from F. L. Vogel and J. N. Zemel

A NOVEL material has just made its debut which provides an artificial counterpart to natural petrified materials which preserve the fine detail of the original perishable structure. Natural petrified wood for example, is formed when the original wood is infiltrated by silicious matter which converts it into a stony substance preserving the original structure. There are no comparable processes used industrially that convert organic materials to a more permanent form. The recent paper by T. L. Ward and R. R. Benerito (*Thin Solid Films* 53, 73; 1978) is therefore particularly interesting. They describe the unusual transformation of ordinary cotton fibre into a glassy material reminiscent of the permineralisation of wood.

In the Ward-Benerito process, cotton fabric is first soaked in a sodium plumbite solution after which it is dried, placed on a glassy substrate and heated to temperatures as high as 600 °C. During heating, the treated cotton initially carbonises to a black colour. As the temperature is raised, it picks up silicious material from the substrate and becomes yellow. At 600 °C the carbon skeleton is impregnated with silica and is converted to a glassy substance reproducing details of the original fabric. The authors demonstrated the results of this process with many artefacts including labels and decorations on bottles, adhesives between glasses and free standing thin films.

These results are interesting as they indicate the existence of a novel material. Still more interesting perhaps is the origin of the process which produces it. Ward and Benerito examined the composition of the plumbite-treated material using a variety of analytic techniques such as X-ray diffraction, optical and electron microscopy (TEM and SEM), ESCA, EDAX, DTA, X-ray fluorescence and atomic absorption. These studies indicated that lead was uniformly distributed in both the unheated and the transformed cotton. Furthermore, it was observed that the residual carbon concentration in the charred cotton

was six carbon atoms to one lead atom. Since lead is not known to form a carbide, this result may be taken as an indication of an interstitial or intercalation compound described in a recent popular review (Fischer & Thompson *Phys. Today* July, 1978). This conjecture is supported by the following observations.

When cellulosic materials are charred, a high defected hexagonal layered structure forms. While this is not a true graphite, it may be sufficiently similar that some degree of intercalation is possible.

The process of interstitial insertion can in itself improve the crystal perfection of the hexagonal layered structure resulting in a closer approach to a graphitic material.

The relatively low temperature of the process suggests that the diffusion occurs by an interstitial mechanism.

Silicates are known to form layered compounds, one common example of which is mica.

The mechanism suggested by this combination of facts is that intercalation of lead separates the spacing between the roughly formed hexagonal layers sufficiently to allow interstitial diffusion of the silicious materials from the substrate.

Neither the 'petrified cotton' nor the process to produce it arose in response to a stated need. As such, it is a fascinating example of a fundamental study of a technical substance. Since long term stability of cellulosic materials such as wood, paper and cotton is notoriously poor, it might be expected that permineralisation of these substances by the Ward-Benerito technique could be used. Diverse examples of this would include document preservation, converted wood as a decorative building material, or lacy *objets d'art* that could be produced no other way. As with any new material, uses will probably be found that cannot be foreseen at this early stage.

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His modest description of his work as the correction of a series of errors was accompanied by his strong claim for the importance of his work on the transmission of information from DNA to the site of protein synthesis by long-lived RNA molecules. His statements on this subject are to be found

in his Weizmann Memorial Lecture, *The Biological Role of the Nucleic Acids*, delivered in April 1959 and published in 1960. The discovery of soluble RNA and microsomal RNA did not solve what "for the geneticist and immunologist [was] the major problem", wrote Brachet, "the mech-

anism of specific protein synthesis." Such a mechanism could be visualised if it were assumed that the "nucleus produced an intermediary substance involved in protein synthesis; there is little doubt that this intermediary is RNA...". In contrast to the *Escherichia coli* model with a short-lived messenger, Brachet's model, based on the alga *Acetabularia*, necessarily assumed a long-lived messenger, because of the long duration of protein synthesis in enucleated fragments, and this model represents more closely than the *E. coli* model, the situation in higher organisms.

Reading Brachet's Weizmann Lecture today one is struck by the scattered distribution of specific statements on the nature of 'template' (that is messenger) RNA. This may in part account for the lack of appreciation of these *Acetabularia* studies in the history of molecular biology.

The relation between chemical structure and morphological organisation was explored in several papers, most notable of which was the one by Jacques Roger of the University of Paris. He outlined the early history of the concept of an 'organic substance' as a state of chemical combination responsible for vital phenomena independent of any morphological organisation in the anatomist's and microscopist's sense of the word. The concept of a glutinous, mucilaginous, albuminoid, and later a colloidal substance can, Roger claimed, be traced back to this organic substance. Equally, the claim that the chemist can study this substance to the advantage of our understanding of vital phenomena can be traced back to eighteenth century writings on the organic substance—those of Bourguet, Buffon, Rouelle, Venel and Lamarck. In other papers the importance of the recognition of definite limits to the miniaturisation of morphological structures was stressed. Maxwell in 1875 was the first to assert that the dimensions of protein molecules set a lower limit to the postulation of such organised particles as the germules introduced by Charles Darwin in his hypothesis of pangenesis. Fantini emphasised the novelty of the concept of three-dimensional, specific structure in macromolecules determined by sequence, and he contrasted this with the vaguer conception of shape to be found in colloid science.

The establishment of this Research Group in Italy is an important event in the history and philosophy of science. Hitherto work in the subject has tended to be dominated by Anglo-American activities. The programme initiated by the Research Group should not only act as a catalyst to the growth of the subject in Italy, but should also stimulate such work in the area of the

life sciences, as befits a group associated so closely with the international activities of the Zoological Station. □

Globular clusters

from Virginia Trimble

THE globular clusters comprise the oldest stellar population readily available to us for study. They ought, therefore, to be able to provide the answers to various questions in galactic and stellar formation and evolution—if only we knew which questions to ask. The chief difficulty lies in deciding whether they are the rare (about 10^{-4} of the observed mass in the Universe) survivors of a population of once-common, fundamental building-blocks from which larger units (galaxies and clusters) have arisen, or the products of early fragmentation in previously-existing gas clouds that we might call protogalaxies.

Uncertainty on this basic point underlay and sometimes befuddled discussions of new observations and calculations at a recent NATO Advanced Study Institute on Globular Clusters.* For instance, the average heavy-element abundance of a population of globular clusters increases with the mass of the galaxy to which it is attached (S. van den Bergh), and, within at least the Milky Way and the Andromeda Galaxy, abundances range systematically from nearly solar at the centre, through a steep decline 5-10 kpc out, to one-twentieth of solar or less in the halo (with large scatters at all positions; L. Searle). But is this trying to tell us something about how star-formation rates (and, therefore heavy element production) depends on gas density or something about how dynamical friction juggles units of varying mass and degree of central concentration?

Dynamics of stars within individual clusters can be considered almost without regard to their origins, because at least the inner parts are relaxed. The identification of seven X-ray sources (including five bursters; W. Lewin, G. Jernigan) with globular clusters lends extra urgency to calculations of core evolution and collapse (L. Spitzer), although recent estimates of the surface area required to produce the bursts and the relative timing of optical and X-ray emission in one burst now strongly suggest that the source is an accreting neutron star rather than a massive black hole formed by core collapse. Core evolution may still have

to be invoked to put a neutron star into a suitable X-ray-producing binary system, as it becomes increasingly clear that the clusters are deficient (relative to stars in the galactic disk, at least) in binary systems over a considerable range of parameters (J. Gunn, R. Griffin, M. Liller, E. Budding). But all is not lost, even if core collapse never occurs (J. Katz), as it may be possible to get the observed X-ray flux from single neutron stars occasionally passing through the stellar winds of red giant stars (A. Fabian). The amount of ambient gas implied by this is well below the 0.1-1.0 M seen or set as upper limits from HI and HII studies of several clusters (G. Knapp, W. Liller). Some of these limits are, however, uncomfortably low for standard theories of mass loss from evolving stars and gas escape from clusters (D. Faulkner).

Progress has been made on several traditional, vexing, rather technical issues. The wide giant branch in ω Cen is undoubtedly to be attributed to real abundance variations, and because CN line strength is generally correlated with Ca, Fe, and Sr abundances (J. Norris), the variations must have been intrinsic to the gas from which the stars formed, and not just produced by mixing within individual giants. Real variations in CNO abundances occurring among and within other clusters (especially 47 Tuc; A. Rodgers), are not always uniquely correlated with variations in Fe and heavier elements (J. Frogel, E. Persson), and may also be primordial (R. Kraft); but they are not the whole answer to the traditional 'second parameter' problem.

The second parameter(s) is whatever is responsible for the lack of the theoretically-expected correlation of the appearance of a cluster's HR diagram with its heavy element abundance. The most conspicuous effect is the occurrence of both red and blue horizontal branches in metal-poor clusters, especially in the outer parts of the galaxy (R. Zinn), when only blue ones would be expected. High CNO (either primordial or mixed up from the core) would cure this, but CNO cannot deal (V. Castellani) with a more subtle effect seen in some far-halo clusters (R. Schommer) in which the red-giant branch is too red for the metal abundance implied by the main-sequence turn-off point and integrated cluster spectra. HR diagram morphology is also affected by helium abundance, cluster age, mass loss from red giants, and stellar rotation. It will probably be necessary to include the contributions of at least two 'second parameters' to match the full range of observed HR diagrams. Rotation is apparently also important (A. Sweigart) in finally making it possible to under-

*Held on 7-18 August at the Institute of Astronomy, Cambridge.

stand how processed material can be mixed up from the hydrogen-burning region to the surface of low mass stars by means of meridional circulation.

No consensus now seems possible on the meaning (or, in some cases, even the reality) of colour and abundance gradients within clusters other than ω Cen (K. Freeman; M. Chun; G. Da Costa). The question of whether or not the absolute brightnesses of RR Lyrae variables are correlated with their metal abundances is 'controversial.' It is important because these stars are one of our standard candles, whose brightness partially determines the extragalactic distance scale, and so Hubble's constant and the age of the Universe. W. Liller

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and V. Clube strongly advocated a relationship (partially based on recent photometry by B. Carney) in which the RR Lyraes in clusters of lowest metal abundance are about one magnitude fainter than those in clusters of high Z. A constant brightness for these stars, independent of cluster composition, was equally strongly supported on both observational and theoretical grounds by P. Demarque, R. Kraft, and F. Hartwick.

The various problems of globular cluster astronomy are probably in as good shape as we can expect, considering that (although some of the clusters were recorded by Messier) the first classification of them by radius and central concentration was begun only about 50 years ago by H. Sawyer Hogg, who was an active participant at the Institute. $\square$

infected cultures showed the presence of about three copies of non-integrated hybrid genome per cell. Some copies might have integrated with the host DNA and been maintained as such but the evidence for this was not conclusive. Infected monkey cells, which with wild type SV40 support the production of mature virus, were found to contain amounts of nonintegrated, superhelical hybrid DNA which was stably propagated when the cells were cloned.

There are several advantages to this system of nonlytic propagation using a crippled vector. First, the recombinant DNA is never expressed as an infectious virus particle simply because it does not possess the necessary genes. This satisfies some of the safety considerations involved in this type of work. Second, since assembly of a complete particle is not needed, the amount of DNA that can be 'carried' on the SV40 vector is probably increased, and third, the host cells remain viable, which offers the chance of long term studies or the maintenance and expression of 'foreign' DNA sequences in a mammalian system.

Hamer *et al.* looked for expression of the bacterial gene in the original lytic infection and found that although the sequence was transcribed into RNA, no functional suppressor tRNA was detectable. This is not surprising in view of the different types of processing of tRNA that probably take place in bacterial and eukaryotic cells (Knapp *et al. Cell* **14**, 221; 1978; O'Farrell *et al. Nature*, **274**, 438; 1978). This may not be a problem when the gene 'insert' is from a more closely related donor. Rumour has it that correct transcription and expression of globin genes cloned in an SV40 vector in mammalian cells has been achieved in Paul Berg's laboratory at Stanford.

There are a variety of sequences that could usefully be worked on in a system of this kind, but many of them will probably have to wait until more has been done to reduce the possible hazards of working with a tumour virus. The vector in these experiments could still transform cells, and it may be necessary to remove this ability before the SV40 is sufficiently 'crippled' to use with genes that might confer a selective advantage on the hybrids.

Other routes exist for getting genes into mammalian cells, for example, by cell fusion and selection (Szybalski *et al. Natn. Cancer Inst. Monogr.* **7**, 75; 1962; Littlefield *Science*, **145**, 709; 1964) and such genes may be expressed in adult animals (Illmensee *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 1914; 1978). But in terms of simplicity and flexibility the tumour virus vector could be a winner. $\square$

Bacterial gene into monkey cells

from John Jenkins

ONE of the long term aims of genetic engineering recently came a step closer to reality, with the stable propagation in mammalian cells of a hybrid DNA molecule containing a mammalian tumour virus chromosome and a bacterial gene. The successful development of such vectors for introducing and propagating genes in mammalian cells offers the hope of eventually being able to study the control of expression of eukaryotic genes in a system not too far removed from their natural environment. Even further in the future is the possibility of using such vectors to introduce particular genes to correct inborn genetic defects.

The idea itself is not new. In 1972, Jackson *et al. (Proc. natn. Acad. Sci. U.S.A.* **73**, 2904; 1972) described the construction of a hybrid molecule from simian virus 40 (SV40) and bacteriophage λ DNA, and such hybrids can replicate in suitable host cells (Ganem *et al. Cell* **7**, 349; 1976). The problem is that replication of the hybrid virus kills the host. A new development gets round this problem by tailoring or 'crippling' the SV40 so that the genes responsible for cell death are cut out before the hybrid is made.

SV40 can behave in two different ways when it infects a cell, and its genome can be divided into two regions on this basis. In rat cells, the entire virus genome is inserted into the host

chromosome and the cells change many of their growth characteristics. This transformation requires the expression of only about half the SV40 genome making up what is known as the early region. The second type of infection, of monkey cells for instance, requires expression of most of the genome. In this case, the remainder of the viral genes are functionally expressed and about 36 h after infection mature virus particles appear, followed by the lysis of the host cells. Cell types that support this lytic cycle of infection are termed permissive, while those which transform but fail to produce mature virus are called nonpermissive.

In order to cut out the genes responsible for lytic infection, (Hamer *et al. J. molec. Biol.* **112**, 155; 1977) cleaved the circular virus DNA at two points within the late region, using the restriction enzymes *HpaII* and *EcoRI*. The large fragment from this, containing the origin of DNA replication and the early region genes, was ligated to a DNA fragment containing an *E. coli* tRNA suppressor gene. In order to get large amounts of the hybrid DNA it was necessary to first grow it up in monkey cells in the presence of a helper virus which possessed functional late region genes. Purified hybrid DNA from this lytic cycle was then used in the present series of experiments (Upcroft *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 2117; 1978).

The hybrid DNA was found to transform nonpermissive rat cells, and analysis of total cellular DNA from

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Forty years of plant mitochondriology

from A. L. Moore

It has taken approximately 40 years since the first paper was published describing their oxidative capacities for a symposium to be held specifically devoted to plant mitochondria.*

Although the cytochromes of plant mitochondria are spectrally and functionally similar to those of animal mitochondria, there is still confusion about the role of the multiple *b* cytochromes in electron transport. B. T. Storey (University of Pennsylvania, Philadelphia) suggested that cytochromes *b*-556 and *b*-560 are part of the respiratory chain whereas cytochrome *b*-565 only associated with the main chain in the energised state and in the presence of antimycin A. This suggestion was based upon the kinetic behaviour of *b*-565, in particular its complex oxidation/reduction kinetics during coupled substrate oxidation. However a few papers were presented which disagreed with this interpretation and it was suggested that a possible Q-cycle mechanism could explain the complicated nature of *b*-565 reduction under oxidative conditions.

P. R. Rich (University of Cambridge) gave a detailed analysis of the paramagnetic centres associated with higher plant and *poky N. crassa* mitochondria. Of particular importance was the finding that no EPR-detectable component was directly attributable to the alternative oxidase and furthermore it was suggested that there is no evidence for a direct involvement of iron sulphur centres or transition metals in a paramagnetic state in the terminal oxidation system. Although the HiPIP and ferredoxin-type centres are analogous to their mammalian counterparts, an EPR signal characteristic of a high-spin ferric iron with a *g* value at 4.3 was independently reported by Rich and M.-F. Henry (University of Amsterdam). Henry presented evidence in favour of this iron-containing protein's being involved in the cyanide insensitive respiratory pathway although the redox behaviour of this signal under conditions in which the alternative oxidase was operating was questioned. Obviously further work on the function of this component is required before its possible role in alternative oxidations can be better understood.

The topic which generated much interest and led to lively round-table discussions was the relationship between the malate oxidoreductases and NADH dehydrogenases. The pro-

cess of malate oxidation is much more complex in plant than in animal mitochondria. Malate can be oxidised by two malate oxidoreductases and the NADH produced may be oxidised by at least four different NADH dehydrogenase systems. One of these dehydrogenases is located on the outer surface of the mitochondrial inner membrane.

Although there is general agreement that the oxidation of malate is mediated by an NAD⁺-linked malic enzyme and malate dehydrogenase, the exact location of these enzymes is still controversial. J. M. Palmer (University of London) took the view that the oxidation of malate can provide NADH for oxidation by both the internal and external NADH dehydrogenases and that malate reduces external NAD⁺ primarily by the activity of externally located malic enzyme and malate dehydrogenase. In contrast, however, J. T. Wiskich (University of Adelaide) supported the view that the external NADH dehydrogenase was not involved in the oxidation of malate in the absence of added NAD whereas in its presence a transmembrane transhydrogenase has been postulated to operate, which is considered to transfer reducing equivalents from internal NADH to external NAD thus connecting internal malate oxidation to the external dehydrogenase. D. A. Day (University of California) presented evidence on the malic enzyme distribution in potato mitochondria from which he concluded that malic enzyme is not present in the intermembrane space of these mitochondria and furthermore that all malate oxidation occurs in the matrix. Perhaps in this respect, the most interesting findings reported at this symposium were the detailed investigations of M. Neuburger (Université de Grenoble) on the transport of NAD⁺ through the inner membrane of purified mitochondria. Convincing evidence was presented which indicated that highly purified intact plant mitochondria possess a NAD-translocator. Most workers readily agreed that the existence of such a carrier would explain many of the conflicting reports in the literature on malate oxidation and the further characterisation of this carrier is eagerly awaited.

The sessions on the molecular and cellular aspects of cyanide resistant respiration attracted considerable attention. The exact nature of the alternative oxidase has remained elusive for many years. However, as W. D. Bonner (University of Pennsylvania, Philadelphia) pointed out, a considerable amount of evidence has accumulated which allows a fuller understanding of its branchpoint from the main respiratory pathway and the nature of the products of the alternative oxidase with

oxygen. Bonner suggested that a modified protonmotive ubiquinone cycle satisfies the kinetics of cytochrome interactions, and that the alternative pathway accepts electrons non-protonmotively from this cycle. This proposal suggests that a pool of ubiquinone in the succinic dehydrogenase region of the respiratory chain is performing as the branchpoint for the alternative pathway. Bonner concluded that the alternative oxidase could either be a species of ubiquinone or an EPR-silent metalloprotein that facilitates the reaction of the ubiquinone species with oxygen. In this regard a great advance in our knowledge of the nature of the alternative oxidase was obtained from the studies of Rich and S. Huq (Imperial College, London) who presented results on the isolation and partial purification of the alternative oxidase from *Arum maculatum*. Both workers used a hydroxamic acid-sensitive quinol oxidation as an assay for the oxidase. Remarkably, Rich found that in a deoxycholate solubilised preparation the quinol oxidase activity remained very stable, which seems to suggest that the lability of the oxidase in intact mitochondria may be due to the connection between the dehydrogenases and oxidase, and not the alternative oxidase itself. Both preparations contained minimal amounts of cytochromes *aa₃*, *c* and *b*. It was of particular interest to note that the overall product of oxygen reduction by the quinols was water.

A. M. Lambowitz (University of St. Louis) reviewed the current status of the biosynthesis of the alternative oxidase in *Neurospora* mitochondria. An important development in this area has been the isolation of antimycin-sensitive mutants with defects in the biosynthesis of the alternative oxidase. All cytochrome system activities seem unaffected in the mutants and the only apparent defect is in the induction of the alternative oxidase. Lambowitz suggested that the results indicate that there are at least two polypeptides unique to the alternative oxidation system. □

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Erratum

In the article 'Halogenated hydrocarbon effects' (*News & Views* 274, 533; 1978), in the last paragraph, column 1, on page 534, the first line should read "Both the PCBs and the polybrominated biphenyls (PBBs) induce hepatic effects". In the second paragraph, column 2, line 4 ff. should read "Poland . . . believes this to be a receptor for TCDD and consequently for other inducers of aryl hydrocarbon hydroxylase . . .".

*The First International Symposium on Plant Mitochondria was held at the Luminy campus in Marseilles on 31 July-4 August.

review article

ASSESS II: a simulated mission of Spacelab

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ASSESS II, a joint exercise of NASA and ESA, was set up to enable scientists to become accustomed to an atmosphere-free, gravity-free working environment. This simulation of Spacelab conditions has provided new data and a valuable insight into the possibilities and limitations of such a mission.

IN THE 1980s many scientists in Europe and the USA will be able to fly experiments in space using the Spacelab now under development by the European Space Agency (ESA). The experiments will cover a wide range of disciplines and will often fly on a single mission together with equipment for engineering and industrial applications. It is important for would-be experimenters, and ESA, to learn early those operational techniques specific to Spacelab which will enable scientists to gain maximum benefit from its advantages of an atmosphere-free and gravity-free environment.

In 1975 NASA and ESA conducted a joint exercise, ASSESS I, using a Convair 990 research aircraft, the Galileo II, belonging to NASA's Ames Research Center, in a preliminary simulation of a Spacelab flight. Four scientists were confined to the aircraft for a week, during which six research flights were conducted, with a multi-experiment payload (three USA and three European), aimed at astronomy and stratospheric studies. The results of ASSESS I were encouraging, in that they showed how experimentalists with only a few weeks' specialised training could act as proxy operators (payload specialists) for a range of experiments not necessarily from their own fields of research. However, the project was assembled rather hastily, and was not as realistic a simulation as desired. Experiments were not organised ergonomically into a payload, making the work of the payload specialists exceptionally difficult. No attempt was made to use a common integration site, with specially prepared ground support equipment, and the ADDAS onboard computer system for handling scientific data could not be used satisfactorily because of lack of time to prepare and check software.

ASSESS II, flown in mid 1977, aimed to improve the realism of the simulation, and provide another chance for scientists to acquire new data. The same aircraft was used, with six American and six European experiments on board, run by four payload specialists. The mission was longer, with nine research

flights each of 6-h duration carried out in a 10-d period. Payload specialists were confined within the aircraft, or in a juxtaposed sleeping trailer, communicating to the outside world only by telephone or radio. Here we describe how the mission was organised, and present some of the initial data from the European experiments.

Choice of experiments

The ESA selection committee based its choice of experiments to fly on (1) its scientific value; (2) the state of experimental readiness; there was just over a year between experiment selection and flight, so it was preferable to use an experiment which did not have to be built up from scratch; (3) its ease of operation: using payload specialists to run three experiments each, some highly complex, meant avoiding apparatus with many fine tuning adjustments, switches to throw continually, and widely separated pieces; and (4) the similarity to the types of experiments envisaged for Spacelab.

The full complement was chosen for mutual compatibility to avoid, as far as possible, clashes in the mission timing and trajectory plans. The selected experiments were: A threefold infrared astronomy experiment, based on a 32-cm infrared telescope (Meudon). This carried a continuum photometer for observing interstellar dark clouds (Groningen), and a line spectrometer for interstellar sulphur in HII regions (Garching); a television camera to study infrared airglow from noctilucent terrestrial clouds (Southampton); a sub-millimetre spectrophotometer to measure the Sun's chromospheric brightness temperature (Naples-Florence); a LIDAR system to measure aerosol distribution in the troposphere (Oberpfaffenhofen); medical probes to study diurnal and stress-induced behaviour of the payload specialists (Bad-Godesberg); and test equipment to study the electromagnetic environment, and help remedy earthing problems (Noordwijk). (This was operated by the crew and used also on behalf of the USA payload.)

Italian, French, Dutch, German and British groups were represented as for ESA satellite programmes, and as intended on Spacelab.

Experiment preparation

Build up of the ASSESS II experimentation was begun at experimenters' home institutes in late 1976; the payload specialists received two to three weeks' initial training on

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12. ESA-SPICE, Linder-Höhe, Porz-Wahn, FRG.

experiment in this period. In January 1977 all the experiments were gathered at the ESA/SPICE facility at the DFVLR, Porz-Wahn near Köln. Here they were integrated electrically and mechanically into a mock-up of the aircraft, and simulation tests of the European half of the payload were carried out. It was convenient for the principal investigator teams to attend for long periods, but the payload specialists who were on the spot all the time increasingly handled the equipment. During this period they were able to write their detailed procedures for installing and operating the experiments on the aircraft.

The simulation was very realistic, demanding continuous manning of the experiments and of a control room designed and operated as a replica of that at Ames. Two sets, of four 'flights' each, tested the skills of the payload specialists, and their interaction with the ground teams. After this simulation the experiments were moved to California, where the whole (including USA) payload was put together on the ground at NASA Ames, then installed in the aircraft and flown.

The data flights

In late May 1977 the simulation mission of nine flights was performed, followed by a set of 'principal investigator flights', during which the Spacelab-like conditions were relaxed, and more personnel went on board to assist with each experiment. The nine simulation flights took place on schedule with minimum intervals of less than one day for maximum loading on the payload specialists. Communication during the mission was via a control centre, in which the teams running the flight and payload operations lived. The scientists on the ground could

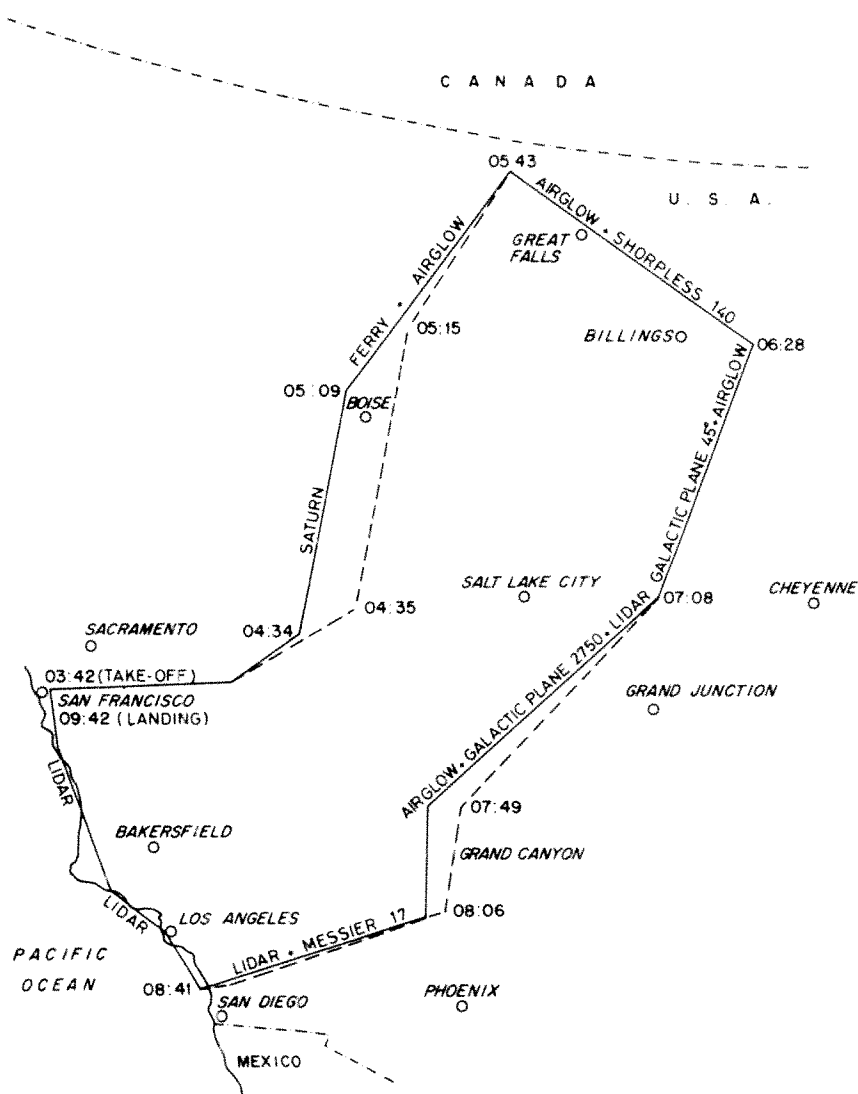
talk to the payload specialists for limited periods, about two hours per day, to advise on experiment preparation in the light of information from the previous data-take. A TV camera enabled some visual information to be passed 'upwards' to the aircraft (only available when on the ground) while a regulated allowance of data was removed from the aircraft between flights, corresponding to a bit rate which can be accomplished by planned telemetry from the shuttle. In practice communication during actual flights was difficult—this was one way in which conditions were significantly worse than those anticipated for Spacelab. Another difference was the successive takeoffs, landings, and refuellings, with their disruptive effects. Figure 1 shows a typical trajectory for a night flight, and gives an idea of the distribution of activities for the European experiments during such a flight. The LIDAR and the solar experiment were kept running for most sections of all the trajectories, providing routine data on the Earth's atmosphere. Two night flights over the Pacific viewed southerly astronomical objects, and the day flights included prolonged manoeuvres over cities and peculiar terrains, for LIDAR and for the USA payload, which was mainly Earth-resource orientated.

The individual experiments, and their results will be presented elsewhere. Here we indicate the organisational complexity of the project, and the payload specialists' tasks.

Infrared astronomy

A 32-cm aperture infrared telescope which had flown on ASSESS I, carried two instruments, a 4-band photometer spanning the wavelength range 31–196 μm , and a Fabry-Pérot

Fig. 1 The trajectory of an ASSESS II night-time flight over the Western USA. Data were taken throughout each 6-h flight, while preparations and post flight checks took between 6 and 10 h longer. Flight tracks for Purple 1, 21 May 1977: solid line, original plan; dashed line, revised plan; all times UT.



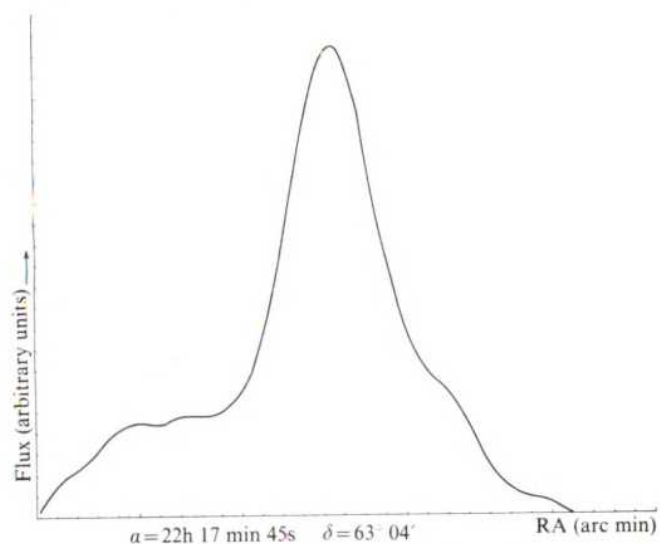


Fig. 2 A scan of the dark interstellar cloud Sharpless 140, obtained with the Groningen photometer in the waveband 115–196 μm (TII filter). The abscissa registers a scan amplitude of 29 arc min in RA using a diaphragm of 4.5 arc min diameter.

line spectrometer, both cryogenically cooled. The payload specialist could switch between them using a flip-flop mirror in the telescope. A sensitive TV camera facilitated finding and locking onto infrared position, by serial spot-follower and gyro-stabilised control loops. These experiments were run throughout the mission by one payload specialist.

Figure 2 shows a typical output scan; new flux maps of the molecular clouds S140(A), R Cr A, and M17 were built up from such scans, and previous measurements of the galactic plane^{2,3} and of sky noise⁴ were revised with increased precision. Spectra at the 33.6 μm wavelength of SIII emission with the Fabry-Pérot system were, however, inconclusive.

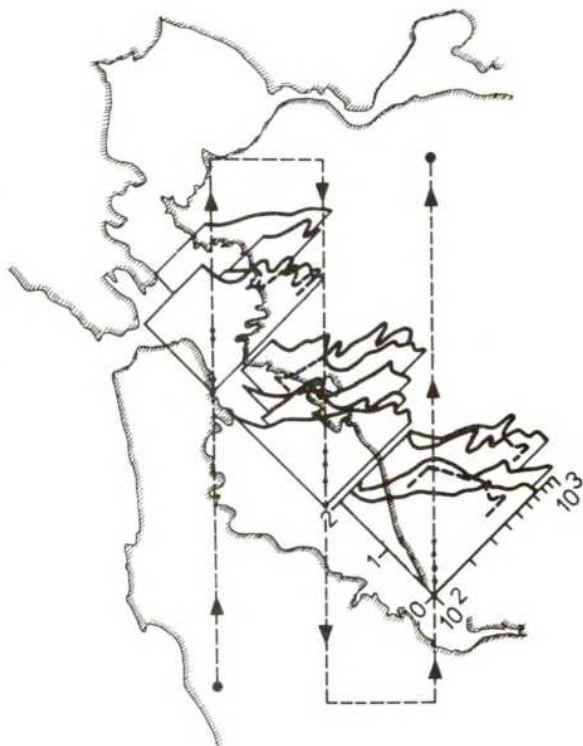


Fig. 3 Mass concentration of aerosol particles (in mg m^{-3}) as a function of altitude over a portion of the San Francisco Bay area, measured by the LIDAR experiment on ASSESS II.

Solar brightness temperature

During daytime flights a Michelson interferometer was fed by a gyro-stabilised coelostat, giving solar spectra in the range 300 μm –2 mm wavelength. The aim was an absolute determination of the chromospheric brightness temperature to within 250 K, for use in physical modelling. The equipment was also operational on all other flights, yielding terrestrial atmospheric emission spectra varying with latitude and altitude, and examining submillimetre sky anisotropy over a 2° field, aimed at the cosmic background radiation⁵.

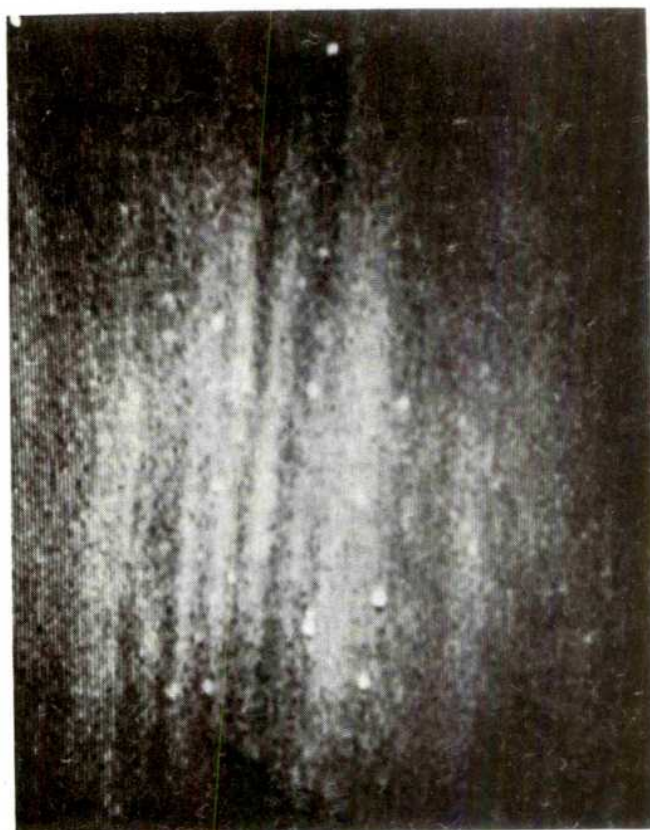


Fig. 4 OH airglow seen in the form of narrow stripes with the TV camera system at 700–900 nm.

Lidar

Figure 3 shows an aerosol distribution diagram for an area south of San Francisco. It shows the effectiveness of the airborne LIDAR, which obtained such data over urban areas (Los Angeles basin and San Francisco Bay) and over very varied terrain (Mojave desert, Rocky Mountains, and the Central Valley of California). The already standard LIDAR technique of measuring the time of flight of back-scattered pulsed near infrared laser radiation emitted from the aircraft, was calibrated using the simultaneous data of Dr P. Russell, taken from the ground at the Stanford Research Institute.

Airglow TV

Mesospheric airglow from the OH layer, ~85 km above the Earth was studied for structure due to winds, atmospheric tides, and gravity waves. The TV technique used was a development of that flown on ASSESS I (ref. 6), recording images in the 700–900 nm waveband every 0.15 s. This compares with 5–10-min photographic exposures from mountain sites^{7–9}. Figure 4 shows narrow-banded mesospheric structures, observed during ASSESS II. During the mission broader bands were commonly seen, also patches, and V-shaped structures. Amid this variety of geometrical patterns, banded structures with tens of km periods and maximum

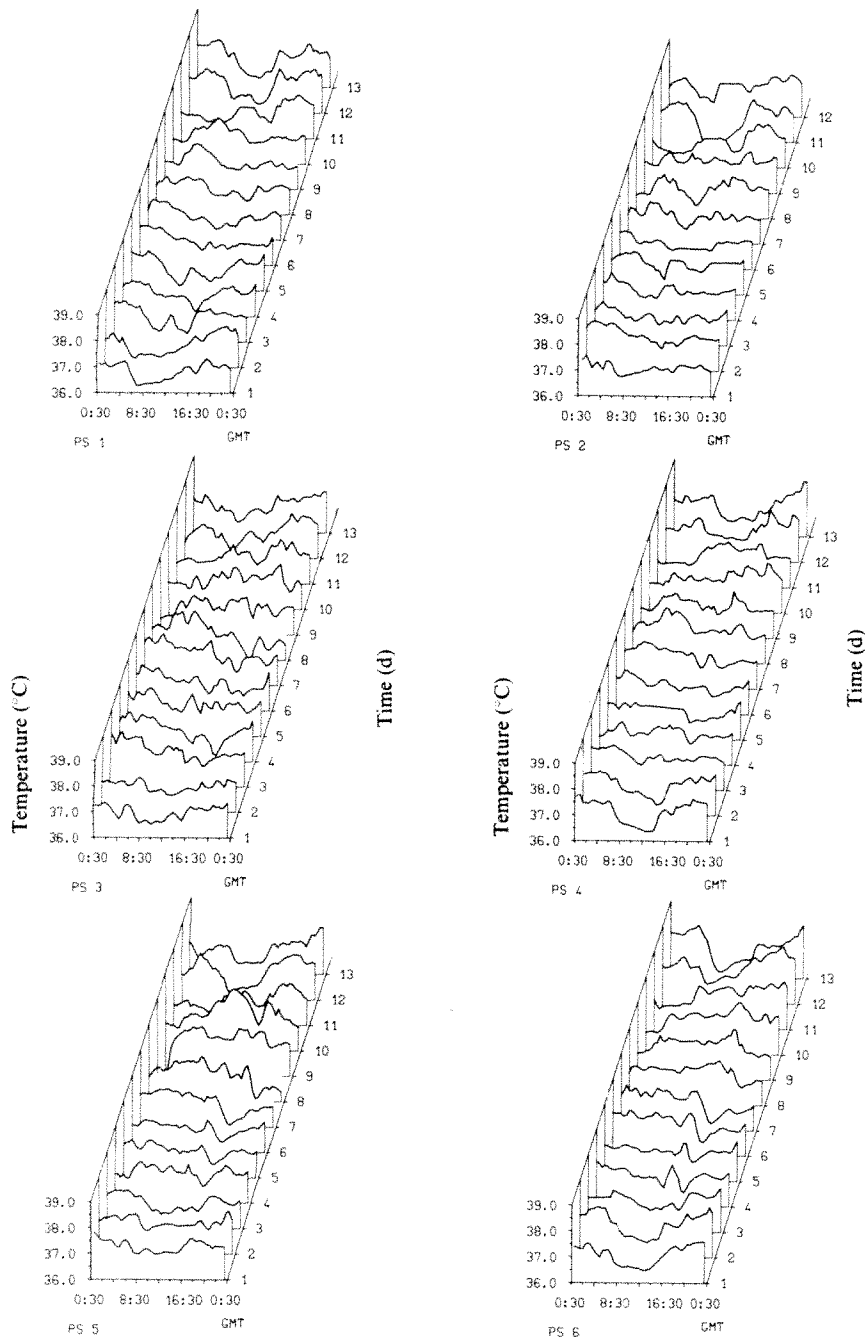


Fig. 5 A survey, abstracted from the medical experiment data, of the temperature rhythms for the six payload specialists (two USA, two European, and two European ground back-up) working on the ASSESS II mission.

extension around 700 km were frequently prominent. The latter seem too small to fit gravity wave predictions¹⁰. The data, a clear improvement on ASSESS I, show that tropospheric storms can cause mesospheric airglow pattern changes, and provide evidence for multiple layers in the airglow, from sets of bands at fixed common angles.

Medical experiment

The medical experiment was aimed directly at Spacelab. Each payload specialist (two Europeans and two Americans flying, plus two European back-ups on the ground) was fitted with a rectal temperature probe, and ECG (electrocardiogram) electrodes, recording continually on to miniature tape-recorders carried on a belt. During sleep the EEG (electroencephalogram) was measured and similarly recorded. Every three hours, except during sleep, a urine sample was collected and stored frozen for subsequent analysis of hormones and electrolytes.

A global look at the temperatures of the payload specialists (PSs) is given in Fig. 5, for two days pre-flight, nine flight days, and two post-flight days. Effects of slipping the working routine

against the 24-h day are seen in the drift of the diurnal maxima and minima, (see PS 1, 5 and 6); the increasingly broken rhythm (see PS 3, also 2 and 4) is due to poor sleep. Full analysis of the 10 parameters recorded will be valuable for planning activities and routines for Spacelab.

Experiment grouping

The infrared astronomy experiments, comprising five experiment racks and the telescope, were run by one payload specialist while the LIDAR, Airglow and Solar experiments were run by the other. Ergonomically this worked quite well, as the latter three were controlled mainly from a centralised panel, and the effort demanded for each group was roughly equivalent. Although considerable progress in reducing trivial switch-throwing had been made since ASSESS I, further automation would have undoubtedly reduced stress on the payload specialists.

Data management

The airglow, medical, LIDAR, and astronomical photometer experiments were either entirely self-contained in data storage

and quick-look analyses or used the ADDAS system only as a back-up. The other experiments relied on ADDAS as their main data-processor; however, time and funding limitations caused experiment-ADDAS interfacing to become a major problem immediately before launch.

Lessons

Some clear lessons have been learned from the ASSESS programme, which can be used as a series of general guidelines for would-be Spacelab experimenters; the most evident are given below.

(1) Keep interfaces with Spacelab simple. This includes the mechanical, the electrical, and signal processing. The latter is particularly interesting, as Spacelab will provide a central data management system (CDMS) analogous to the ADDAS used in ASSESS II. Self-contained data gathering, processing and storage by any single experimenter not only gives him practical flexibility, and eases competition for scarce CDMS memory, but allows him to develop software at his own pace and with his own personnel during the build-up to a flight. The CDMS should be used to send highly selected data by telemetry to the ground, allowing a team to analyse problems for which the payload specialist has no time during the mission.

(2) Electromagnetic cleanliness is important. ESA set precise earthing and shielding standards for the European experimenters. These were not mandatory during ASSESS II for the US experiments, which therefore had far more problems with electromagnetic interference. Failure to adopt such standards could be costly.

(3) Experience is an effective way to learn. Those experiments which had been aboard ASSESS I gave a data yield nearly an order of magnitude greater when flown this time. This indicates that airborne missions are highly cost-effective ways for scientists to improve their potential rewards from Spacelab flights. Similar criteria apply to the management teams who feel that their direct experience on ASSESS would be valuable in organising Spacelab.

(4) For payload specialist efficiency unified control panels for groups of experiments must be built up. Diverse experiments will therefore need to be integrated, on the ground, into a single payload and also to check-out cross-talk effects, and because any payload work on board the shuttle will be costly

and complex. From a European viewpoint the build-up of a team of engineering experts at an integration site ('SPICE') within an ESA member state was shown to be practical. This centre gave a much better training scheme for payload specialists than the method used during ASSESS I, of sending payload specialists only to experimenters' home institutes for training purposes.

(5) Time and capability for *in situ* diagnosis and repairs to experiments in flight is very limited, when one payload specialist may have care of around 20 separate payload elements. A useful approach is to predict the probability of key failures, and build in redundancies as well as using modular experiment design, with replaceable key back-up units. In spite of problems, the value of the payload specialists was shown by the quantity and quality of the data obtained on ASSESS II, and clearly, using the shuttle with payload specialists will enable many more experimenters to use space platforms, with a much wider range of experiment categories than can participate in conventional satellite programmes. One result of ASSESS II is to show that the comparable complexity needed to build and operate airborne experiments, the lead times required, and the relationship to laboratory instrumentation, make aircraft programmes very suitable preparation and training for Spacelab. This is true for the experimenters, for the payload specialists, and also for the management teams. Whether ASSESS I and II have provided enough experience for NASA and ESA managements, and whether or not this is used, only time and the Spacelab programme can tell.

We thank staff members of NASA Ames Research Center, Informatics Inc., NASA Marshall Spaceflight Center, NASA Kennedy Space Center, NASA Headquarters, ESA-SPICE, DFVLR Porz-Wahn, ESA Headquarters and ESA-ESTEC who contributed to the success of this mission.

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articles

Formation of the early Earth regolith

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Appropriate physical conditions for the origin of life could exist on the relic regolith grains. A regolith of this type could be formed on the entire surface of the Earth under the effect of space factors in the early epoch which was characterised by a strongly rarified atmosphere. In the subsequent epoch with a denser atmosphere and suitable conditions for the first occurrence of liquid water droplets such grains had to acquire a considerable biochemical activity.

EXISTING hypotheses about the origin of life on the Earth are based either on the initial conditions of the planet or on those of space, and can be subdivided into two distinct groups. The first such group assumes that the bioevolution started in primitive water bodies¹—especially in the course of submarine volcanism², whereas the second group supports the concept of the formation of prebiological material on the surfaces of interstellar dust grains which were later transported into the Solar System and to the Earth^{3,4}. The hypotheses of the first and especially of the second group have been severely criticised^{5,6}. However, several objections can be avoided if a new hypothesis

were considered based on the following conditions necessary for 'the origin of life platforms' (developed and formulated in refs 1, 5, 7): (1) large specific area of grains (with appropriate macro- and micro-porosity); (2) immersion of grains into the water medium; (3) free energy available for biochemical reactions; (4) global character of the appropriate conditions; (5) large (in the geological sense) time span for the events; and (6) absence of powerful inhibiting factors. None of the existing hypotheses satisfies these conditions completely.

We put forward here a hypothesis with initial conditions that cannot fully be related either to the first or to the second type of hypotheses and which are 'on the border' between planetary and space conditions. According to our hypothesis, the regolith grains which formed on the early Earth's surface during an epoch of a strongly rarified atmosphere could serve as the platforms for the origin of life. We designate this type of regolith as relic or *R*-regolith. In contrast, an airless planet's regolith (such as the Moon's) will be designated *M*-regolith. We shall show that the conditions for the formation of each of these two types of regoliths are different.

Together with cosmic electromagnetic and corpuscular irradiation, meteorite bombardment is known to be one of the main exogenic factors responsible for the formation of a planet's regolith. We believe that the most interesting situation arises on a planet almost completely lacking an atmosphere ('nearly atmosphereless'). In this case the bulk of a micro-meteorite flux reaches the planet's surface with an appreciable loss in the mass and velocity of particles whose final distribution function $I_f(m_i, v_i, P)$ is transformed from the initial case $I_i(m_i, v_i, P)$ so that in the atmosphere pressure (P) range, from $P_1 \sim 10^{-7}$ atm to $P_2 \sim 10^{-4}$ atm, mostly small particles with velocities $v_i < 10^5$ cm s $^{-1}$ impinge on a planet's surface⁸. When $P > P_2$, $I_f \rightarrow 0$, extremely quickly, whereas at $P < P_1$, ($I_i - I_f \rightarrow 0$) but much more slowly. However, the penetration of space electromagnetic and corpuscular radiations through a rarified atmosphere is significant. For instance, a CO₂ atmosphere with $P \approx 10^{-5}$ atm (which has a penetrability equivalent to a 6.5×10^{-3} cm thick CO₂ solid filter) is rather 'transparent' to the fluxes of UV and X-ray radiations and insignificantly detains charged particles with energy $E > 0.1$ MeV (ref. 9).

Hence it seems that the regolith-forming conditions on the surface of a nearly atmosphereless planet will be different to those on an atmosphereless one. Hence, a different regolith forms on each of these planets.

It is interesting to consider the problem of *R*-regolith formation in conjunction with the investigations of Meadows¹⁰ and others which establish the existence of an atmosphereless period on the Earth (after loss of the primary H₂-He atmosphere) which was followed by a period of gradual accumulation of a new atmosphere. At first, the new atmosphere consisted mainly of CO₂ and H₂O vapours—the products of mantle degassing and volcanic activity. Since then the present atmosphere has been developing. More than 4,000 Myr ago there was a crucial moment in this evolution. When the atmospheric pressures reached $P \approx 10^{-3}$ atm and the Earth's surface temperature became $T \approx 273$ K (triple point for water), the appropriate conditions for liquid H₂O occurrence were created¹¹. Before this, water desublimation resulted only in the formation of low mobile hard ice mounds and in hoarfrost. The epoch of smaller atmospheric pressures and temperatures, directly adjacent to this period, satisfied the conditions necessary for *R*-regolith formation. Therefore, we assume that by the time that water condensed, the Earth's surface was covered with an *R*-regolith which predetermined the directions of geochemical and prebiological evolutions.

Let us consider certain physical properties of *R*-regolith and, primarily, its macroporosity determined by an average grain-size distribution which, in its turn, is determined by the correlation between the rates of the two competing processes of comminution and aggregation. As for *M*-regolith, the process of comminution in *R*-regolith occurs under the impacts of micrometeorites, whereas aggregation results from cohesion

and agglutination. In a rarified atmosphere the specific energy of an impact $E_i = v_i^2/2 \approx 10^9$ erg g $^{-1}$ is sufficient only to break up the grains, and not to melt them ($E_m \approx 10^{11}$ erg g $^{-1}$ (ref. 9)). Consequently, the *R*-regolith will not contain glassy particles and glassy surfaces which are characteristic of the *M*-regolith¹². On the other hand, gas from a rarified atmosphere is immediately adsorbed on the split-off surfaces of grains which decreases drastically their aggregation due to the deterioration of the cohesive properties of grains by several orders of magnitude. Therefore, the competition between comminution and aggregation will favour the former. Consequently, the average grain-size distribution in *R*-regolith will decrease, whereas its specific surface S_R and hence its macroporosity will increase from the minimum value $S_M \approx 0.1$ m² g $^{-1}$, characteristic of *M*-regolith¹³, by one or two orders of magnitude.

Microporosity should be regarded as another peculiar feature of the *R*-regolith. Micropores result from the radiational and structural defects (tracks, dislocations, and so on) in the volume of the grains. The presence of micropores in the grains of *M*- and *R*-regoliths is due to the absorption of hard cosmic electromagnetic and corpuscular radiations. However, such defects are hardly detected in the Moon's regolith because the micropores in its grains are continuously destroyed by high-velocity micrometeorite impacts (which cause shock waves in the grain volume and melt grain surfaces) and due to sharp diurnal fluctuations of the surface temperature¹³. This has been confirmed by simulation experiments when the Moon's regolith was irradiated¹³. This situation is very similar to the conditions existing on a nearly atmosphereless planet.

The *R*-regolith micropores had to contain gas resulting from the photodecomposition of atmospheric gases (dissolved in the volume of grains) as well as rock- and mineral-forming oxides. This gas in micropores had to be in a physically adsorbed state and enriched with O₂ which accounted for about 50% of the specific weights of most rocks, minerals and some atmospheric gases.

Now, let us consider the conditions of chemical evolution which existed on the Earth's *R*-regolith during the time of liquid H₂O condensation on the planet's surface. The gas accumulated in the micropores of the grains and adsorbed on the macropores, had to escape outside by the Rebinger effect (adsorptional decrease in strength¹⁴) and by displacement desorption with water, which has the greatest energy of desorption ($E_a = 10$ –12 kcal mol $^{-1}$) relative to other common gases ($E_a = 3$ –8 kcal mol $^{-1}$ (ref. 9)). By releasing gas from the pores of *R*-regolith, water partly occupied its place and in the near-surface atmosphere, probably, initially O₂ and then other gases (CO, N₂, and so on) were evolved. However, the atmosphere remained fairly transparent to UV and other space radiations which penetrated into the pores of the *R*-regolith grains and served as a source of free energy for photo- and radiochemical processes. Thin water layers around the grains did not significantly hamper the penetration of UV and corpuscular radiations. For example, a 2 cm thick layer of water is pervious to 50% of UV radiation with $\lambda = 1,880$ Å (ref. 9), while the range of UV radiation penetration in to the grains depends on the kinds of rocks and minerals and the degree of their porosity.

The conditions (1), (2) and (3) were very favourable in the *R*-regolith for various chemical reactions to take place and different radically from those existing on the unsaturated surfaces of *M*-regolith grains. Indeed, *M*-regolith grains are cleaned by solar radiation in vacuum to an 'atomically-pure' state. Therefore, only chemisorption (that is, adsorption of a monomolecular layer) can occur on their surfaces and the grains are characterised by a low adsorption capacity¹⁵. Only simple molecules can be formed on *M*-regolith grains (for example, H₂ on the Moon's regolith¹⁶).

On the other hand, distinctive characteristics of *R*-regolith grains such as a developed system of macro- and micropores and great adsorption capacity make them resemble the interstellar dust grains despite the different origin of these

properties in the latter¹⁷. Although the precise chemical composition of the interstellar dust grains is not yet known, from the results of Hoyle, Wickramasinghe¹⁷⁻¹⁹ and others, we suppose that many have a silicate matrix, and that all are covered with a polymeric mantle resulting from chemical evolution. The interstellar dust grains in this evolution played the part of a third body providing the necessary energy and momentum balances in the chemical reactions. A similar role could be played by *R*-regolith grains. All this suggests an analogy between the conditions of chemical evolution on the interstellar dust and *R*-regolith grains. Over 40 molecular compounds, including some important for prebiological evolution such as HCN, and H₂CO, have been recorded in interstellar gas-dust clouds¹⁸. In our opinion, the conditions for complex chemical reactions to occur in the *R*-regolith of the Earth were more favourable and promoted a more rapid chemical evolution than in the interstellar gas-dust clouds owing to a higher (10¹⁰–10¹² times) density of the ambient gas around the particles, significantly greater concentration of the *R*-regolith grains, relatively higher temperature ($T \approx 273$ K), and the presence of water medium. Indeed, if the grain surface is considered as a saturated gas then the rate ψ of chemical reactions, occurring on a grain surface surrounded by the ambient gas with the sticking coefficient equal to unity, will be proportional to n/t , where t is a characteristic time of the chemical reaction. At sufficiently high temperatures of the regolith grains (T_R), t can be found from the Arrhenius law: $t \sim \exp(-E_{act}/T_R)$, where E_{act} is the energy of activation ($E \leq 5$ eV) and T_R is the temperature in eV ($T_R \approx 0.04$ eV). The non-exponential dependence $t_d(T_d)$ is characteristic of the interstellar dust grains with their low temperature $T_d < 10$ K. According to Goldanski³, t_d may be constant $\approx 10^{-2}$ s, hence $\psi_R \gg \psi_d$.

These conditions could not only promote the formation of at least analogous and, apparently, even more complex compounds on the *R*-regolith but, what is more important, at considerably greater rates than on the interstellar dust grains. Moreover, the branching surfaces of the *R*-regolith grains acted as sites for the replication of complex linear molecular structures, whereas complex stereoregular structures were formed in the water medium in the pores (the conditions are described in ref. 20). For example, the surfaces of grains could accelerate the evolution of coacervates¹ which could be formed in the pores of the flooded *R*-regolith grains.

Hence, having compared the conditions in the interstellar dust and in *R*-regolith, one might confirm that the chemical evolution on the *R*-regolith could probably advance over a geologically short period to prebiological and biological forms, because of the fulfillment of conditions (1), (2) and (3). Conditions (4) and (5) were also present on the Earth at that time,

because the *R*-regolith covered the entire planet's surface uniformly and existed for a 'geological' period of time. The accumulation of an atmosphere created the conditions for the wind transfer of the *R*-regolith grains and, consequently, for the transportation of biological forms. At the same time, this atmosphere (as it accumulated and because oxidising) together with the increasing layer of water above the *R*-regolith grains could screen the action of the inhibiting factors. Note that unlike other hypotheses about the origin of life on planets our's does not indicate the existence of powerful inhibiting factors. For instance, having got into the water, a porous *R*-regolith grain could float for a certain time until most of its pores filled with water. That period of time might be sufficient for some prebiological reactions to occur in the grain whose products, together with the grain, could sink stepwise to the bottom of the pools to safely continue evolution there.

In conclusion, the conditions in the *R*-regolith during the epoch of water condensation satisfy the generally accepted conditions (1)–(6) necessary for the primary forms of life to have originated on Earth. It can also be supposed that such conditions have not been realised on Mars. The present status of the martian regolith is close to that of the Earth's *R*-regolith before the start of water condensation²¹.

Of course, the probability of finding the remains of the 'buried' *R*-regolith is extremely slim but its reproduction in laboratory conditions seems a possibility.

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Andesitic volcanism and crustal evolution

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Petrological evidence indicates that andesite is a mixture of melts from the upper mantle and lower crust. Mixing is favoured by a compressional environment and generation of rhyolitic crustal melt is favoured by continental crust. Andesitic volcanism apparently involves both addition of new material to the crust and fractionation of pre-existing crust.

A CRITICAL problem in crustal evolution is whether the voluminous andesitic volcanoes and cogenetic granodioritic batholiths are derived from the mantle, crust, or both. If these

rocks originate within the crust, they represent recycled crust. If they originate within the mantle, they represent new crust. Where they occur within the ocean basins, most commonly as the andesitic island arcs of subduction zones, they represent the generation of new continental-type material in an otherwise basaltic crustal environment.

Andesite and granodiorite do not occur alone, but belong to the basalt-andesite-dacite-rhyolite association and its plutonic equivalent, the gabbro-diorite-granodiorite-granite association. Despite the enormous range in composition and physical properties of magmas represented by these rocks, field relationships demonstrate that they are intimately related in time and space. The implication of a genetic relationship among

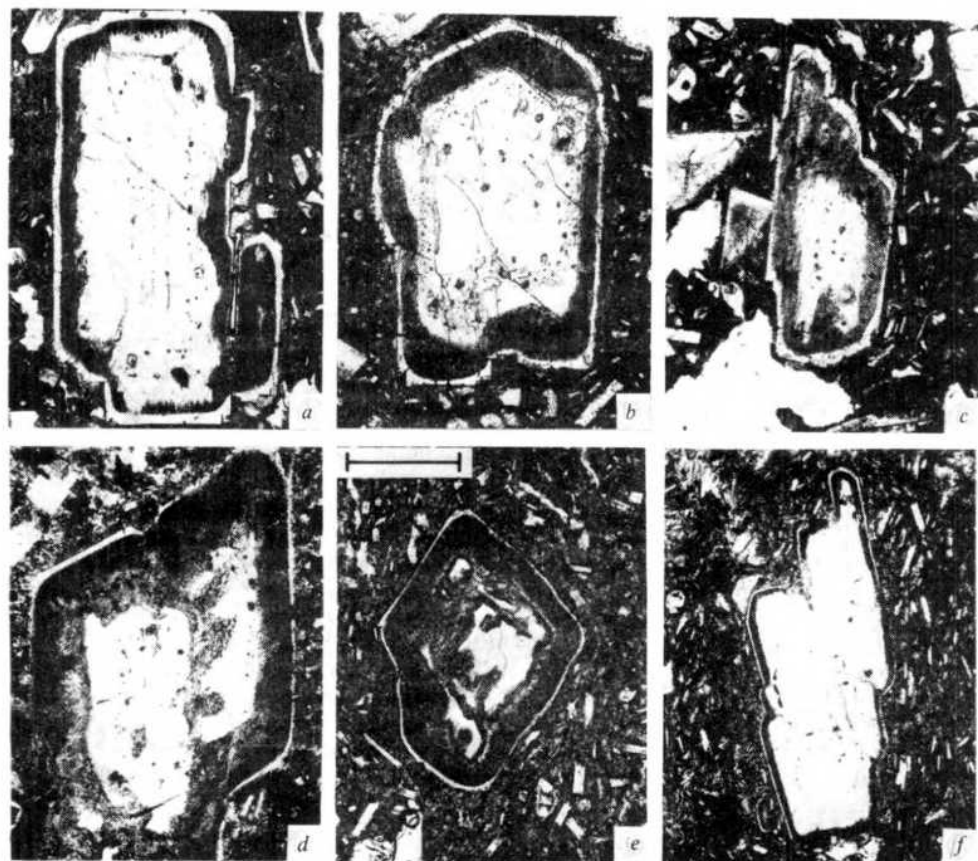


Fig. 1 Resorbed sodic plagioclase in some andesites and dacites. *a*, Glacier Peak (Washington) dacite: An 38 core, An 68 inside overgrowth, An 50 outside overgrowth. Two inclusions of rhyolitic glass (Table 2) are visible in core. *b*, Pitons du Carbet andesite (Martinique, French West Indies): An 52 core, An 75 inside overgrowth, An 56 outside of overgrowth. *c*, La Soufriere de Guadeloupe (French West Indies) andesite: An 54 core, An 83 inside overgrowth, An 57 outside overgrowth. *d*, Bill Williams Mtn dacite (San Francisco Volcanic Field, Arizona): An 23 core, An 55 inside overgrowth, An 41 outside overgrowth. *e*, Nevado Antizana dacite (Ecuador): An 25 core, An 60 inside overgrowth, An 50 outside overgrowth. *f*, Tauhara dacite (Taupo Volcanic Zone, New Zealand): An 20 core, An 61 inside overgrowth, An 46 outside overgrowth. Scale bar 0.5 mm. See Table 1 for additional data.

these magmas is strong. This relationship is a key both to the origin of these magmas and of continental crust. The purpose of this article is to discuss new data on phase assemblages in volcanic rocks (Table 1) which show a remarkable similarity among a variety of igneous complexes of this magmatic association, and which point toward mixing of basaltic and rhyolitic

magmas as a dominant mechanism by which intermediate members of the association develop. The new data include representative andesites and dacites of the Cascade Range of western United States, Taupo Volcanic Zone of New Zealand, Andes of Ecuador, and Lesser Antilles (Guadeloupe and Martinique) of the French West Indies.

Table 1 Phases in andesite and dacite derived from basaltic and rhyolitic magmas

	Volcano	Sample description	Rhyolite-derived		Basalt-derived		
			Pl*	Qz†	Pl‡	Ol§	Xenolith
ISLAND ARC Lesser Antilles	La Soufriere Guadeloupe	Px andesite, summit dome	54*	Yes	91	82	Pl-Px
	Pitons du Carbet Martinique	Hb andesite, ~10 km N, Ft de France	52	Yes¶	92	84	Pl-Hb
	Diamant Martinique	Px andesite, near R. du Diamant	45	Yes	90	84	Pb-Px
CONTINENTAL ARC Cascade Range	Mt Baker Washington	Px andesite, cleaver, Eaton Glacier	46	No	82	70	Pl-Px
	Glacier Peak Washington	Hb dacite pumice, Suiattle River	38¶	Yes¶	81	81	Pl-Hb
	Mt Rainier Washington	Px andesite, Muir Camp Trail	37	No	75	70	Pl-Px
	Mt Hood Oregon	Hb andesite, White River campground	44	No	80	—	Pl-Hb
		Px andesite, Timberline Lodge	—	No	—	79	Pl-Px
	Crater Lake Oregon	Px andesite, Mazama, W. rim Crater L.	36	No	70	74	Pl-Px
	Mt Shasta California	Px andesite, Avalanche Gulch	41	No	81	78	Pl-Px
	Lassen Volcanic National Park California	Hb rhyodacite, Chaos Crags	27¶	Yes¶	92	79	Pl-Hb
		Px dacite, Lassen Pk, 1915 eruption	30¶	Yes¶	83	83	Pl-Px
		Px andesite, Prospect Pks, saddle	31¶	Yes¶	80	80	Pl-Px
	Nevado Antizana Ecuador	Hb dacite, Papallacta L., recent flow	25¶	Yes¶	—	79	Pl-Hb
		Hb dacite, eroded ridge near Papallacta L.	35	Yes	71	—	Pl-Hb
MID CONTINENT Interior west-USA New Zealand	Tauhara New Zealand	Hb dacite, Burrows Quarry	20¶	Yes¶	79	82	Pl-Hb
	San Francisco Volcanic Field, Arizona	Hb dacite, Bill Williams Mt	23	Yes	—	—	Pl-Hb
	Jemez Mountains New Mexico	Ol andesite, Vent 188	20¶	Yes¶	—	84	—
		Px andesite, St Peters Dome	30	No	67	74	Pl-Px
		Ol basaltic andesite, TA-33, LASL	26¶	Yes¶	—	85	—

Pl, plagioclase; Px, pyroxene; Hb, hornblende; Qz, quartz; Ol, olivine

* Composition of core of resorbed plagioclase (Fig. 1) in mole % An.

† Indicates presence of quartz phenocrysts (Fig. 2).

‡ Composition of most calcic plagioclase in mole % An. Occurs as individual grains and within basaltic (cognate) xenoliths.

§ Composition of olivine in mole % Fo. Occurs as individual grains and within basaltic (cognate) xenoliths.

|| Dominant phases of basaltic (cognate) xenoliths (Fig. 3).

¶ Indicates presence of rhyolitic glass inclusions in phenocrysts.

Phase assemblages in andesite and dacite

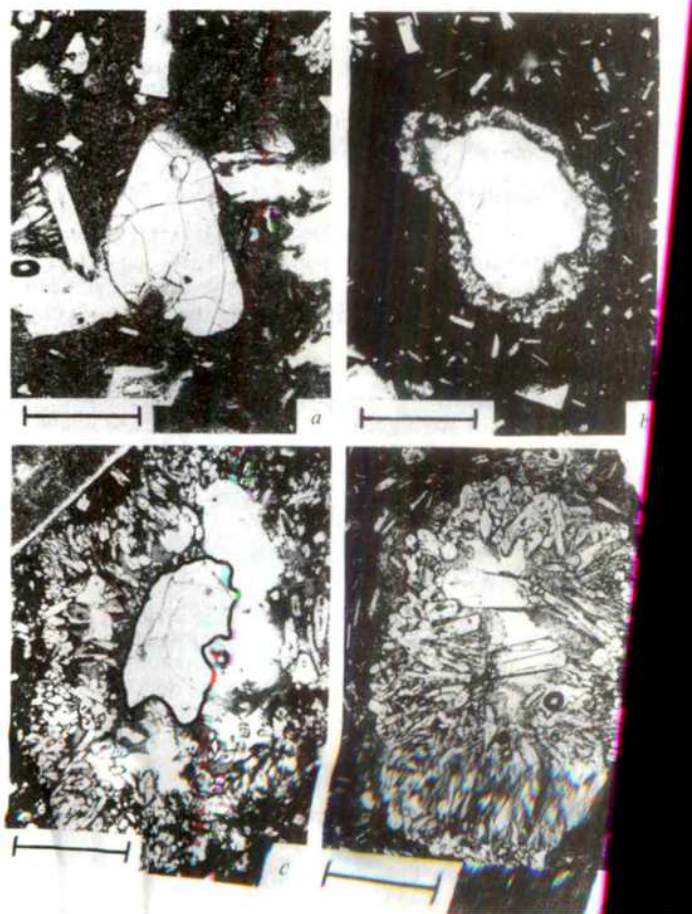
A particularly interesting feature of intermediate volcanic rocks is that they commonly contain phases totally out of chemical equilibrium¹⁻⁷ and of a type and composition appropriate to basaltic and rhyolitic magmas. In addition to the phenocrysts appropriate to magmas of intermediate bulk composition, andesite and dacite contain both anomalously calcic and sodic plagioclase, forsteritic olivine, and sometimes quartz. Sodic plagioclase occurs as cores of phenocrysts and is surrounded by a dark zone of skeletal calcic plagioclase with glass-bearing channels, which is in turn surrounded by a normally zoned clear overgrowth (Fig. 1). The width of the dark zone tends to be greater in phenocrysts in andesite than in dacite. Quartz phenocrysts in dacite commonly contain substantial embayments, indicating resorption. Quartz phenocrysts in andesite are commonly surrounded by glass and pyroxene jackets, indicating resorption and reaction (Fig. 2). Inclusions of rhyolitic glass occur both in the sodic cores of plagioclase phenocrysts and in quartz phenocrysts (Table 2). Forsteritic olivine and calcic plagioclase occur as individual grains and also within crystal-rich xenoliths and microxenoliths of distinctive texture and composition. Inclusions of basaltic glass within forsteritic olivine and calcic plagioclase have been reported by Anderson⁷.

The crystal-rich xenoliths range in size from clots of a few grains present in most thin sections (and individual crystal grains derived from them) to globules 1 m in diameter and are commonly referred to as 'cognate'. They are dominantly plagioclase + pyroxene + glass in andesite and plagioclase + hornblende + glass in dacite. Their texture (Fig. 3) consisting of euhedral to subhedral crystals with interstitial glass suggests advanced crystallisation of a magma. Plagioclase and hornblende in the xenoliths are strongly zoned, acicular, and often hollow, suggesting rapid growth¹⁵. Composition of the hornblende¹⁶ closely coincides with the composition of hornblende coexisting with melt in basaltic systems¹⁷ from 1,000 °C to 700 °C. Bulk composition of the xenoliths is also magmatic, falling at or near the basaltic end of the trend for the complex¹⁸ (Table 2). Those xenoliths which are intermediate rather than basaltic in composition contain sodic plagioclase and sometimes quartz, as described above. Large xenoliths are often concentrically zoned, with a coarse-grained basaltic core surrounded by a fine-grained basaltic or andesitic rind¹⁹. The textural relationship is clearly one of chilling of the xenolith against the host.

These features can be explained in the following way. The composition of the sodic plagioclase and quartz phenocrysts in andesite and dacite indicate that these phases originally grew in a rhyolitic magma. This conclusion is supported by the inclusions of rhyolitic glass within these phases. The composition of the calcic plagioclase and forsteritic olivine indicate that these phases grew in a basaltic magma. This conclusion is supported by the presence of these phases within the basaltic xenoliths and by the occurrence within these phases of inclusions of basaltic glass. This suggests that many andesites and dacites are hybrids derived by mixing of basaltic and rhyolitic magmas. Basaltic magma intrudes rhyolitic magma and breaks into globules. Mixing of liquids occurs at the interface between globules and host. The globules undergo rapid crystallisation to form crystal-rich basaltic xenoliths with andesitic rinds while crystalline phases in the cooler rhyolitic host undergo reaction or melting, forming embayments or pyroxene rims on quartz and dark resorbed zones on sodic plagioclase. These melting and reaction effects are greater in mixtures containing a higher proportion of basalt (andesite versus dacite)¹⁹. Renewed crystallisation following the mixing event results in growth of phases appropriate to the new intermediate bulk composition and temperature of the system, both as individual crystals and as overgrowths on basalt- and rhyolite-derived phases. Overgrowths protect the disequilibrium phases from further reaction. Note that the data presented in Tables 1 and 2 come from both oceanic and continental regions.

Other hypotheses have been advanced for the origin of these features in andesite and dacite. Sodic plagioclase and quartz in andesite have been attributed to assimilation of granitic rocks²⁰. These phases, calcic plagioclase, and forsteritic olivine have also been attributed to crystallisation of andesitic liquid at high pressure, dry in the case of sodic plagioclase²¹ and quartz^{22,23} and wet in the case of calcic plagioclase²⁴ and olivine²⁴. The clear, volcanic nature of sodic plagioclase and quartz (Figs 1 and 2) suggests that they are direct precipitates of magma. Further, the low ⁸⁷Sr/⁸⁶Sr of most andesite is inconsistent with significant contamination by older granitic rocks²⁵. Limited capacity of magmas to assimilate cold rocks and occurrence of andesite in island arcs lead to the same conclusion. This has caused many workers to favour the high pressure crystallisation hypothesis, but this leads to mutually exclusive conclusions concerning water content of the magma. Wet, high pressure conditions might produce the calcic plagioclase but not the sodic plagioclase. Such conditions might cause forsteritic olivine to precipitate but not quartz. It is also difficult to reconcile the high pressure hypothesis with evidence that there is isotopic as well as chemical disequilibrium between phenocryst and matrix in andesite and dacite^{26,27}. Basaltic xenoliths have been interpreted as cumulates^{9,11}, although they lack cumulate textures, and breakdown products of high Al amphiboles²⁸, although they range in size to several centimetres or even tens of centimetres. None of these alternative hypotheses seem to be consistent with the observations presented above, especially the inclusions of rhyolitic glass in sodic plagioclase and quartz and the textures of the xenoliths.

Fig. 2 Stages in resorption and reaction of quartz in dacite and andesite. *a*, Resorbed quartz in Glacier Peak dacite. Note two inclusions of rhyolitic glass (Table 2), one of which has leaked. Scale bar 0.5 mm. *b*, Thin, fine-grained clinopyroxene jacket on quartz in La Soufriere de Guadeloupe andesite. Scale bar 0.5 mm. *c*, Thick reaction zone of clinopyroxene + glass surrounding quartz (outlined for clarity) in Diamant andesite (Martinique). Scale bar, 2 mm. Note large resorbed plagioclase in upper left corner. *d*, End product of quartz-liquid reaction. Clinopyroxene + glass clot in Tauhara dacite. Scale bar, 2 mm. See Table 1 for additional data.



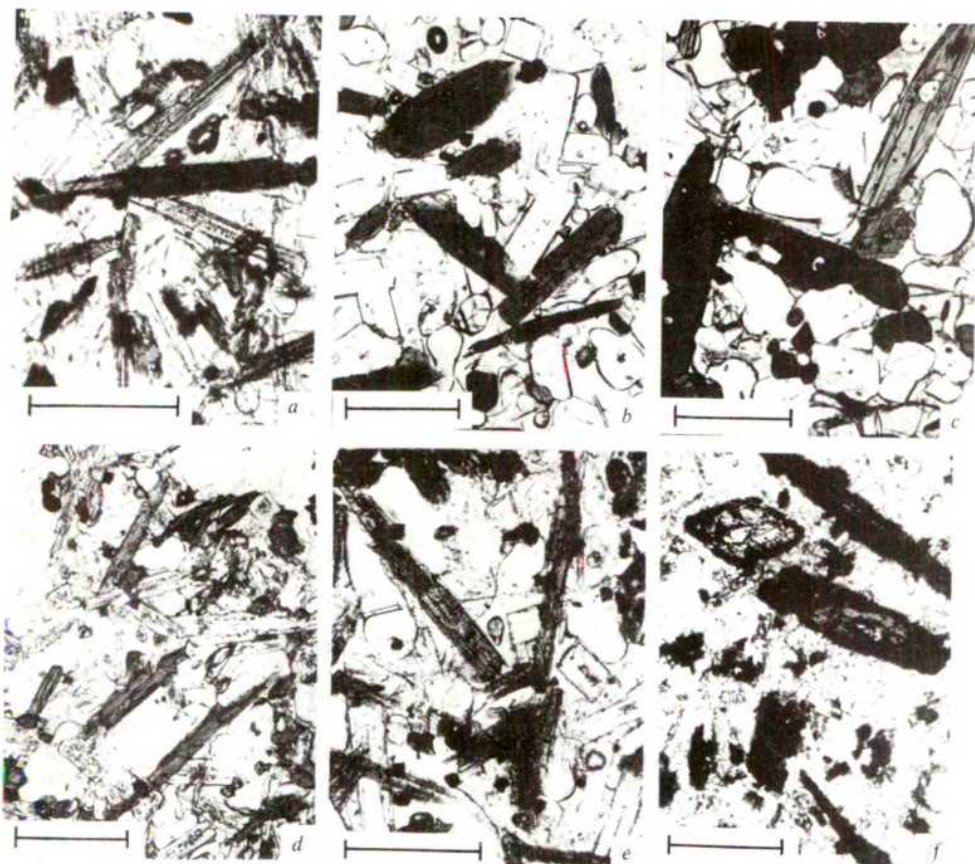


Fig. 3 Photomicrographs of plagioclase-hornblende basaltic (cognate) xenoliths in some dacites and andesites. *a*, Crater Lake dacite (Oregon). Scale bar, 0.1 mm. *b*, Mt Hood andesite (Oregon). Scale bar, 0.5 mm. *c*, Glacier Peak dacite. Scale bar, 0.5 mm. *d*, Chaos Crags dacite (Lassen Volcanic National Park, California). Scale bar, 0.5 mm. *e*, Nevado Antizana dacite. Scale bar, 0.2 mm. *f*, Pitons du Carbet andesite. Scale bar, 0.5 mm. See Table 1 for additional data.

Mixing of rhyolitic and basaltic magmas provides a simple explanation for these petrological features which are both widespread and abundant in andesites and dacites. Mixing can also account for the bulk composition of these rocks, because andesitic and dacitic compositions can be approximated (with some exceptions) as a linear combination of basaltic and rhyolitic compositions^{6,19}. Therefore it seems reasonable to formulate a general model for andesitic volcanism based on this process. Although other processes may contribute to this style of volcanism, consideration of the mixing process leads to important conclusions concerning the relationship of andesitic volcanism to crustal and tectonic setting and to crustal evolution.

Sources of parental magmas

There is general agreement that basaltic magma is a product of partial melting of ultramafic material in the mantle²⁹⁻³¹. However, widespread evidence of mixing in intermediate igneous rocks suggests that the basalt-andesite-dacite-rhyolite suite is not a liquid line of descent from basalt. If rhyolite is not a product of fractional crystallisation of more mafic magmas, it must form directly from a solid source material by a melting process. Rhyolite is too siliceous to be derived by partial melting of ultramafic mantle material or even crustal material at mantle pressures³². It does, however, represent the composition of the low melting fraction of a wide variety of crustal rocks at crustal pressures³³. This suggests that rhyolite forms by partial melting in the crust, just as basalt forms by partial melting in the mantle. The lower crust, which is the hottest part of the crust, is the most probable source region.

As the lower crust is likely to be basaltic (gabbroic) or intermediate in composition³⁴, rather than granitic, rhyolitic magmas must generally form by relatively small degrees of melting of a more mafic source. Relatively low ⁸⁷Sr/⁸⁶Sr of most rhyolites also requires derivation from a mafic (low Rb/Sr) source. The rhyolite source beneath island arcs is most likely basaltic in bulk composition because oceanic crust is formed by basaltic volcanism at the mid-ocean ridges. Experimental studies show that in hydrous conditions in the lower oceanic crust,

basaltic material will be in the form of amphibolite³⁵ and that the melt will be rhyolitic in composition up to about 20% melt fraction¹⁰. Water has a critical role because it stabilises silica-poor amphibole in the presence of silica-rich liquid. The rhyolite source is probably more silicic in the case of continental crust, because silicic lavas on continents tend to be more voluminous and more radiogenic³⁶.

Widespread evidence of mixing also implies close association of basaltic and rhyolitic magmas in time and space. Field relationships attest to this close association as well. Intermediate and rhyolitic rocks are commonly localised within regions of basaltic activity^{19,37}. Hence the rhyolitic parent of these complexes forms in a region of upwards flux of basaltic magma. Because movement of basaltic magma is a most effective means of heat transfer³⁸⁻⁴⁰, it seems likely that basaltic volcanism provides heat for generation of rhyolitic magma in the lower crust and that this is the reason for the close association of basalt with rhyolite in time and space.

Formation of hybrid magmas

The presence in andesite of phenocrysts originally grown in rhyolitic magma implies that the rhyolitic liquid segregates from its source region, coalesces into a magma body, and begins to cool and crystallise before mixing. The common occurrence of granite as large stocks or plutons, the large volume of rhyolitic extrusives, and the large collapse features associated with rhyolitic volcanism indicate that rhyolitic magma moves through the crust as large blobs or diapirs. Large volumes of megascopically homogeneous hybrids suggest that mixtures are formed in large batches. Thus it seems that much of the mixing of basaltic and rhyolitic magmas involves large (often 1 km³ or more) bodies of rhyolitic magma at levels higher and cooler than the lower crust.

Megascopic homogeneity of hybrids also implies an efficient stirring mechanism, probably convection. Application of the Rayleigh-Jeffries criteria to magmas indicates that even the most viscous rhyolitic magmas should convect in most conditions of storage in the crust⁴¹⁻⁴³. Estimated rates of flow in

plutons are of the order of 1 cm s^{-1} (ref. 43), based on the experiments of Peyches and Zorzea⁴⁴ which predict reasonable rates for mantle convection. Injection of basaltic magma into an active rhyolitic pluton favours rapid and possibly turbulent convection^{39,43,45}. The system tends to homogenise itself, driven by the large temperature differences accompanying the initial heterogeneity in composition. Because convection rates are strongly dependent on size of the magma body, the stirring process is effective for pluton-sized pots but not for dispersed melt pockets in the rhyolite source region. This is consistent with the earlier observation that much of the mixing involves large batches of magma.

Field relationships shed further light on how mixing occurs. Volcanic centres which erupt intermediate and rhyolitic magmas lack basaltic vents while they are active, despite frequent basaltic activity in the surrounding region^{19,46,47}. This can be interpreted as indicating that large, active silicic magma bodies beneath these centres trap rising basaltic magma because of the lower density of the silicic magma⁴⁶. Some large volume tephra sheets from andesitic centres seem to record an early stage of mixing in which basaltic magma has been forced to sill out beneath a rhyolitic pluton⁴⁸. In the Crater Lake sheet, silicic tephra is abruptly overlain by mafic tephra. That the parent pluton was mixing, rather than differentiating, is indicated by the presence of quenched mafic material, the xenoliths described earlier, in the silicic tephra.

Statement of model

The foregoing considerations point toward a simple model for the basalt-andesite-dacite-rhyolite association (Fig. 4). Basaltic magma from the mantle rises into the crust and is emplaced at various levels as dikes and sills. In the lower crust, where the initial temperature is high, emplacement of basaltic sills causes partial melting. The resulting rhyolitic liquid gathers into plutons which rise slowly through the crust. Because the rhyolitic plutons exist in a region of upwards flux of basaltic magma, they commonly are injected with basalt and mixed by rapid convection before they crystallise or erupt. Thus basaltic volcanism causes rhyolitic magma to develop and, once formed, rhyolitic magma bodies trap rising basalt. Mixing of the two

parental magmas produces the intermediate compositions, andesite and dacite, of the association.

With this model in mind, comparison of volcanic fields of the basalt-andesite-dacite-rhyolite association in different settings suggests two important relationships: (1) generation of rhyolitic magma is favoured by continental crust and (2) mixing is favoured by a compressional tectonic environment. The first correlation was discussed above and may be due to differences in composition of the rhyolitic source. An example of how this explains variations in andesitic volcanism can be seen in the Tonga-Kermadec-New Zealand magmatic arc. Within this subduction zone, the crust of the over-riding plate varies from continental (New Zealand) to oceanic (Tonga and Kermadec). Silicic lavas of the continental portion of the arc are more voluminous, richer in K, poorer in Ca, and have higher $^{87}\text{Sr}/^{86}\text{Sr}$ values⁴⁹.

The cause of the second correlation is less clear. Extensional or rift environments, as indicated by prominent block faulting, tend to have a bimodal (basalt-rhyolite) lava distribution while intermediate compositions dominate in compressional or subduction environments⁵⁰⁻⁵². Further, changes from compression to extension in the western USA were accompanied by changes from intermediate to bimodal igneous activity^{50,51}. This suggests that extensional tectonic activity inhibits mixing, perhaps because rhyolitic plutons rise more rapidly through the crust where the crust is under tension rather than compression. Where the crustal residence time of active plutons is less, the probability of mixing is also less. Interestingly, in subduction zones, granite is more abundant among plutonic rocks than is rhyolite among volcanic rocks²⁹. Apparently there is a bias towards the eruption of mixtures. Water-rich rhyolitic plutons whose heat is not replenished by injection of basalt are more likely to crystallise at depth as granite, thereby contributing to the silicic batholiths. A further reason for the bias towards eruption of mixtures is that mixing at shallow levels can trigger eruptions by causing vapour exsolution in the magma^{43,45}.

These environmental parameters, continental or oceanic crust, compressional or extensional tectonics result in four categories which determine major characteristics of silicic igneous activity. Continental crust and compressional tectonics

Fig. 4 Model for genesis of the basalt-andesite-dacite-rhyolite association, applied to subduction and rift zone volcanism. In both cases, melting in the mantle gives rise to basaltic magma and melting in the lower crust yields rhyolitic magma. In subduction zones, intermediate lavas predominate at the surface due to mixing of basaltic and rhyolitic magmas within plutons in the mid to upper crust. In rift zones, an extensional environment permits rapid rise of rhyolitic plutons. Both basaltic and rhyolitic magmas reach the surface with little mixing. Except for differences in amount of mixing, the two cases represent a similar response of the crust to heat from the mantle. The rhyolitic component involved in volcanism is larger if the crust is more continental in character.

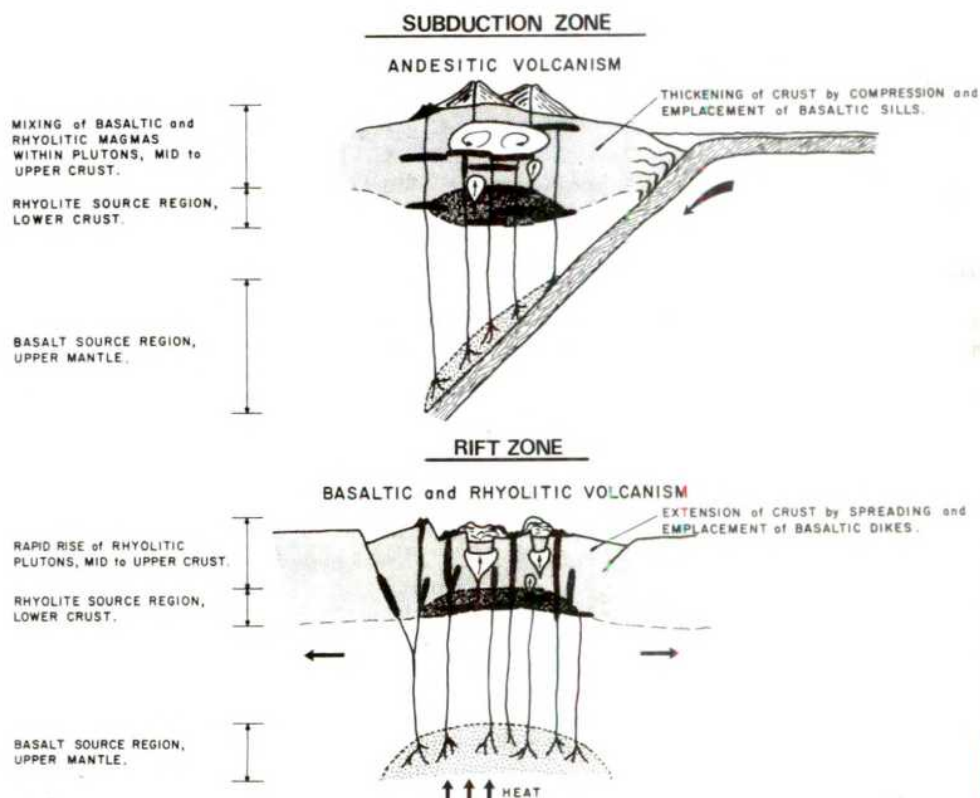


Table 2 Some observed and inferred compositions of continental margin and island arc parental magmas

		Continental margin					Island arc		
		1	2	3	4	5	6	7	8
Rhyolite	SiO ₂	74.3	75.9	75.7	74.8	73.2	73.9	73.8	74.4
	TiO ₂	0.1	0.2	0.1	0.2	0.3	0.3	0.2	0.1
	Al ₂ O ₃	13.3	12.5	11.8	12.0	13.9	12.5	13.3	16.0
	FeO	0.3	0.8	1.0	1.3	2.2	2.3	2.0	1.4
	MgO	0.0	0.2	0.1	0.3	0.4	0.4	0.4	—
	CaO	0.9	0.5	0.7	0.8	1.5	2.3	2.7	2.5
	Na ₂ O	3.8	3.9	3.7	4.0	3.9	4.0	3.9	3.7
	K ₂ O	4.8	5.1	4.2	4.2	4.2	3.2	3.3	1.8
		97.5	99.1	97.3	97.6	99.6	98.9	99.6	99.9
Coexisting plagioclase (An)		20	25		38	35	54		51

		Continental margin		Island arc			
		9	10	11	12	13	14
Basalt	SiO ₂	53.4	48.5	51.4	51.0	49.7	48.7
	TiO ₂		0.9	1.0	0.9	0.5	0.6
	Al ₂ O ₃	19.2	18.0	18.7	18.6	18.0	17.6
	FeO	7.5	8.8	8.6	8.3	10.5	11.3
	MgO	4.9	9.0	5.3	6.3	5.1	5.8
	CaO	9.8	10.7	10.7	11.0	10.0	10.4
	Na ₂ O	2.9	2.3	3.2	2.9	2.4	2.3
	K ₂ O	1.0	0.3	0.5	0.7	0.5	0.6
		98.7	98.5	99.4	99.7	96.7	97.3
Coexisting plagioclase (An)		90	84	90	91	92	85

1, Glass inclusion in resorbed sodic plagioclase, Tauhara dacite, Taupo Volcanic Zone, New Zealand (1 analysis).
2, Glass inclusions in resorbed sodic plagioclase, Nevado Antizana dacite, Andes, Ecuador (5 analyses).
3, Glass inclusions in resorbed quartz, Glacier Peak dacite, Cascade Range, Washington, U.S.A. (4 analyses).
4, Glass inclusions in resorbed sodic plagioclase, Glacier Peak dacite (10 analyses).
5, Rhyolite lava, Medicine Lake Highland, Cascade Range, California (8 analyses; ref. 8).
6, Glass inclusions in resorbed sodic plagioclase, La Soufriere andesite, Guadeloupe, Lesser Antilles (13 analyses).
7, Rhyolite lava, South Aegean Arc, Greece (5 analyses; ref. 9).
8, Melt composition, Picture Gorge Tholeiite, $T = 750^{\circ}\text{C}$, $P = 5$ kbar, H_2O saturated (ref. 10).
9, Basaltic (cognate) xenolith in dacite of Chaos Crags (1 analysis; ref. 11).
10, Basalt of Hat Creek Valley (1 analysis; refs 12, 13).
11, Basaltic (cognate) xenoliths in dacite of Modern Series Santorini Volcano, Greece (5 analyses; ref. 9).
12, Basalt of Main Series, Santorini Volcano (4 analyses; ref. 9).
13, Basaltic (cognate) xenoliths in andesite of Pitons du Carbet, Martinique, Lesser Antilles (2 analyses; ref. 14).
14, Basalt of Martinique (3 analyses; ref. 14).

result in a large rhyolitic component and substantial mixing. The Andes Mountains and Taupo Volcanic Zone are examples. Continental crust and extensional tectonics result in a large rhyolitic component and little mixing. The Yellowstone Plateau and East African Rift are examples. Oceanic crust and extensional tectonics result in a small rhyolitic component and little mixing. Iceland and Easter Island are examples. Finally, oceanic crust and compressional tectonics produce a small rhyolitic component and substantial mixing. Tonga and the Lesser Antilles are examples.

Evolution of the crust

In the model discussed here, andesitic volcanism involves both addition to the crust of new material from the mantle and fractionation of pre-existing crust. With time, these processes of thickening and fractionation would result in evolution from oceanic crust, thin and basaltic, to continental crust, thick and enriched in the low-melting component in its upper portion.

Andesitic island arcs, where thickened oceanic crust is trapped over a heat source, appear to represent an early stage in this evolution. Critical factors which determine whether crustal melting, and hence crustal fractionation, occurs are thickness of the crust, the presence of water, and heat transport from the mantle, either by conduction alone or augmented by movement of magma. Mixing is simply a consequence of crustal melting induced by intrusion of basaltic magma. The higher the conductive heat flow from the mantle the greater the crustal melting accompanying basaltic volcanism. Hence this process of crustal fractionation may have been more efficient earlier in the Earth's history than it is in modern island arcs.

Discussion

This view of andesitic volcanism is a departure from some current thinking on volcanic rocks, but closely resembles models for batholiths^{29,39,53,54}. As such it provides a consistent explanation for the origin of both the volcanic rocks and their

batholithic equivalents. The similarity of these volcanic and batholithic rocks extends beyond bulk composition to many of the petrological features discussed here¹⁶.

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Test of optimal sampling by foraging great tits

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When great tits forage in an unknown environment containing two feeding places of different profitability, they first sample the two places and then exploit the more profitable one. The balance between sampling and exploitation shown by the birds is close to an optimal solution for maximising the number of food-items obtained during a feeding period.

OPTIMAL foraging models, based on the premise that animals collect food in a way which maximises their net rate of food intake, have been quite successful in predicting the decision rules used by predators in laboratory experiments¹⁻⁹, but so far, little work has been done on the problem of how a predator samples the environment. Sampling is an implicit necessity of optimal foraging models, which assume that the predator behaves as if it knows the availability of different prey types or patches of food. We describe here an experiment designed to test whether or not the great tit, *Parus major*, uses a maximally efficient set of rules when sampling a patchy environment.

Two possible methods of obtaining maximal food intake with a choice of foraging area

Our experimental set up is extremely simple. The naïve bird is faced with a choice of two foraging places (patches) which differ

in availability of food. The availabilities remain constant throughout a particular experiment, and the bird can only discover by exploration which of the two patches is better. On the other hand, the bird has distinguished between the quality of the two patches, it should concentrate on exploiting the better one; however, in our experiment we are concerned with how the bird makes the decision about which patch to exploit. We suggest that there are two simple types of maximising rules. On the one hand, the predator might attempt to maximise its rate of intake at every instant in time by always foraging in the patch with the higher expected reward rate. We refer to this strategy as 'immediate maximising'. Alternatively, the predator might attempt to maximise its intake over the total foraging time by sacrificing short-term gain in order to acquire more information about the relative quality of the two patches. In this second strategy, an efficient predator has to choose the appropriate balance between exploration and exploitation. Our main aim is to test whether or not great tits follow this strategy and if so, whether they approximate an optimal balance between exploring and exploiting. The advantage of the sample-then-exploit strategy is that, in contrast to the strategy of immediate maximising, there is no risk of choosing the less profitable patch.

Solution of the 'two-armed bandit' problem
The problem of choosing the optimal balance between exploration and exploitation is similar to the classical 'two-armed bandit' problem, in which the player is faced with two

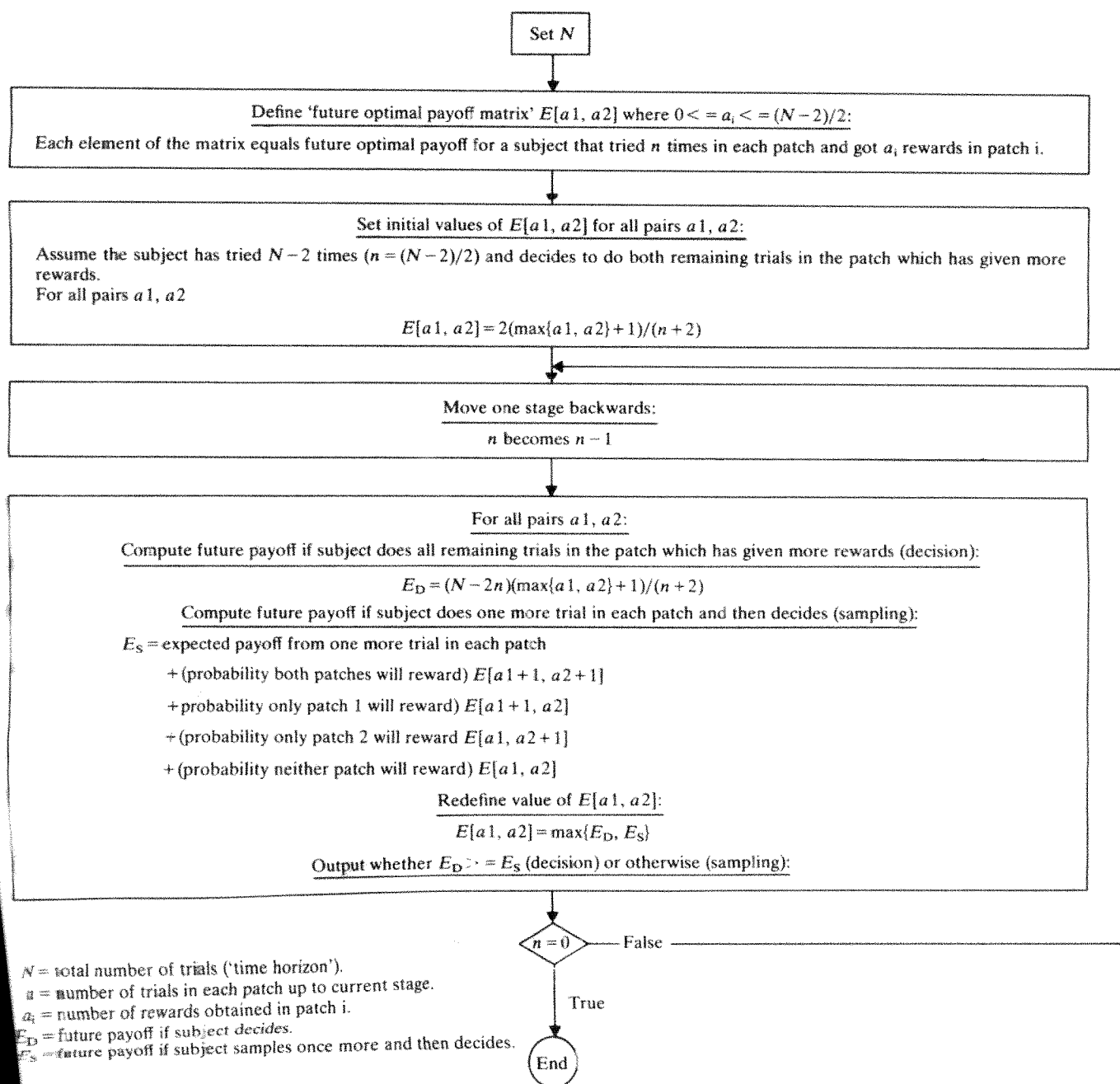


Fig. 1 'Two-armed bandit' simulation.

lines, each with a characteristic, unknown reward rate on a random schedule, and the goal is to maximise the expected number of rewards in N trials^{10,11}. This problem can be solved by a dynamic programming algorithm which works backwards from the N th trial, at which point the machine with the higher percentage rewards is chosen. The algorithm involves analysing each stage, n , the payoff for two possibilities (exploit or exploring) in terms of the known optimal behaviour at stage $n + 1$.

Our model is a modification of the optimal solution of the two-armed bandit problem¹². We consider that the bird has a total of N trials (the meaning of which will become apparent), and at the beginning of an experimental session it goes to each patch alternately. After M trials in each patch it commits itself to one patch or the other, so that all strategies take the form: M trials in each patch (exploration) followed by $N - M$ trials on one patch (exploitation). In our model we added the constraint of equal sampling effort in the two patches during exploration, because this simplifies the model without significantly altering its predictions. The model predicts that

value of M , the point at which the predator decides to exploit, changes with p_1 and p_2 , the reward probabilities in the two patches. Intuitively, one would expect M to decrease as $|p_1 - p_2|$ becomes larger, and our model predicts the exact shape of the curve.

The details of our algorithm, which uses a Bayesian approach to describe the information possessed by the bird at each stage, are as follows. At the start of the experiment, the birds' prior estimate of p (the parameter of the Bernoulli process which is the reward rate in a patch) is considered to be β -distributed with parameters (α, β) , and therefore, with mean $(\alpha + 1)/(\alpha + \beta + 2)$ and variance $(\alpha + 1)(\beta + 1)/(\alpha + \beta + 2)^2(\alpha + \beta + 3)$. The distribution has the property that after n trials with r successes, the posterior estimate of p is also β -distributed with new parameters $(\alpha + r, \beta + n - r)$. Thus, it is easy to follow the state of the bird's information about the two patches by updating α and β after each trial. In simulating the model, we used starting values of $\alpha = 0, \beta = 0$ for both patches. This means that the bird's initial estimate of the mean reward probability in the two patches is 0.50 with a uniform distribution between 0 and 1.0. Thus, after n

trials with a_i successes, the bird's revised estimate of the reward rate p in patch i is $(a_i + 1)/(n + 2)$. We investigated the effect of varying the prior, by calculating our predictions for the following values of α and β : $\alpha = 0, \beta = 2$; $\alpha = 0, \beta = 0$; and $\alpha = 2, \beta = 0$. The model was rather insensitive to changes in the prior, most of the changes in prediction being between 10% and 20%.

If, after n trials in each patch, the bird decides to sample once more, its expected payoff for the remainder of the foraging time is E_s , whereas if it decides to exploit, its payoff is E_D . The optimal strategy is to choose the larger (E) of these two values. E_s can be calculated in terms of the values of E at stage $n + 1$ as follows:

Let

$$\pi_i = (a_i + 1)/(n + 2)$$

Then

$$\begin{aligned} E_s(a_1, a_2, n) = & \pi_1 + \pi_2 + \pi_2 \pi_1 E(a_1 + 1, a_2 + 1, n + 1) \\ & + \pi_1(1 - \pi_2)E(a_1 + 1, a_2, n + 1) \\ & + (1 - \pi_1)\pi_2 E(a_1, a_2 + 1, n + 1) \\ & + (1 - \pi_1)(1 - \pi_2)E(a_1, a_2, n + 1) \end{aligned}$$

and

$$E_D(a_1, a_2, n) = (N - 2n) \max \{ \pi_1, \pi_2 \}$$

Figure 1 gives further details.

The model is simulated by starting with $2n = N$ and working backwards, generating E_s , E_D and E recursively. At each stage, the model produces an $n \times n$ matrix giving the value of $(E_D - E_s)$ for each combination of values of a_1 and a_2 . When the bird is at the last pair of trials, E_D is clearly higher than E_s , assuming there is a difference in the two reward probabilities. As the simulation works backwards, it becomes more likely that, for any particular combination of a_1 and a_2 , the payoff for sampling once more in each patch (and hence acquiring more information about the profitability of the two patches) is higher than the payoff for deciding to exploit. The exact point at which this change from sampling to decision occurs depends on the value of the total number of trials N , which in effect represents the bird's time horizon. For given values of a_1 and a_2 , the larger the value of N , the longer the bird should sample before deciding. We ran the model with a range of values of N .

The experiment

In our experiments, the two patches consisted of two identical operant feeding places at opposite ends of an indoor aviary (measuring 4.3×3.7 m). The feeding places consisted of a perspex disk 35.5 cm in diameter enclosed in a shallow metal box measuring $38.5 \times 38.5 \times 9$ cm. The disk was drilled with 72 holes (diameter 0.7 cm, depth 0.4 cm) around the perimeter of its upper surface. Each of these holes contained a piece of mealworm (weight 0.07 g), and the bird had access to one hole at a time through a small horizontal window in the top of the box which enclosed the disk. To get at the next piece of food, the bird hopped on a perch next to the disk, which operated a solenoid-driven stepping cog to turn the disk through a small angle, bringing the next hole into line with the window. The reward rate was set on a pseudo-random variable ratio schedule using BRD modular logic units. This experimental set up is exactly analogous to that used in probability learning experiments¹³.

The experiments were carried out between June 1976 and February 1977 and involved testing nine wild-caught, adult great tits. Each bird was first trained to perform the operant task and then given a series of tests of different treatments consisting of various percentage reward rates in the two disks. The values we used were 50:0; 40:10; 35:15; 30:20. It is important that although these ratios always summed to 50%, we think the birds did not learn this, as between each treatment they were subjected to a series of one to four 'neutralisation' tests in which both disks offered a 7.5% reward rate. These tests were always continued until the bird showed no marked preference for one

disk over the other; we used a low reward rate during neutralisation sessions because we found this to be effective. If there was a slight preference, the less preferred disk was used as the more profitable place in the next treatment. A 'treatment' (for example, 20:30) consisted of a series of between 1 and 5 10-min tests. We had to run a series of short tests because the birds sometimes emptied one of the disks of rewards before a treatment was completed. When this happened, we interrupted the treatment for a short time while the disk was refilled. During these interruptions the bird was locked outside the test aviary without food, and in our analysis we always discounted the first 20 hops after an interruption. Any particular treatment was continued until the bird had 'decided' to exploit one disk or the other (nearly always the more profitable place).

The decision criterion was that the bird should perform more than 90% of a sequence of 100 hops on the preferred perch. This decision criterion was chosen after calculating the probability of the bird going back to the less profitable perch for a bout of 20 or more hops after different lengths of hopping bouts on the profitable perch. With the 100-hop criterion the probability of this type of reversal is nearly asymptotic and is less than 5%. Each bird was tested twice with all five treatments, the sequence of testing being randomly chosen. At the beginning of each treatment, the test bird was deprived of its normal food and excluded from the indoor aviary while the disks were being loaded with prey. Preliminary trials showed that the birds worked at a constant rate (15 hops per min) throughout a series of tests after an initial 60-min deprivation, which was the minimum period we used. The data were recorded on a computer-compatible event recorder which stored the information on magnetic tape.

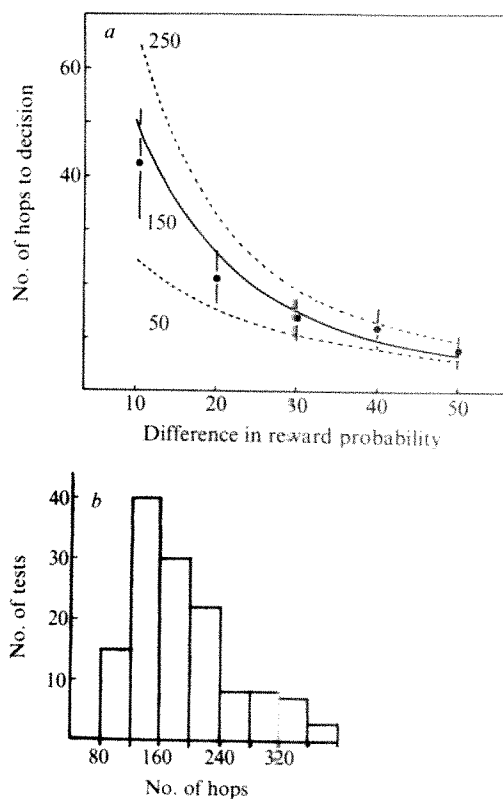


Fig. 2 *a*, The predicted (—, ---) and observed (●) number of hops before making a decision (nine birds). Three predicted curves based on the 'sample-then-exploit' model are shown, for $N = 50, 150$ and 250 . The observed means are close to 150 (continuous line) curve. Also shown are the 95% confidence intervals of the observed geometric means (we used geometric means to normalise the distribution). The x axis is the difference in reward probability between the two perches for the five experiments (for example, 50:0, difference = 50; 35:15, difference = 20). *b*, A frequency distribution of lengths of tests. The modal value is close to $N = 150$, which gave the best fit in Fig. 3*a*. $\bar{x} = 199$.

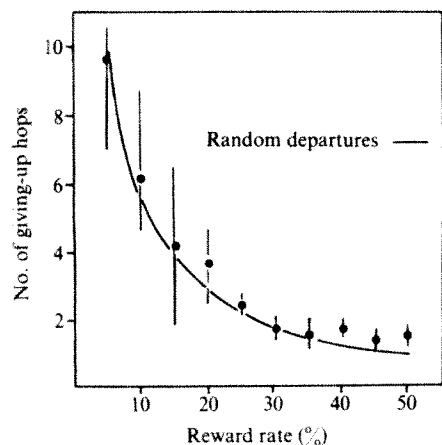


Fig. 3 The observed number of 'giving-up hops' (unsuccessful hops before a departure) plotted against reward probability. The random curve is calculated taking into account the fact that the birds tend to hop in short bouts. If p is the probability of getting a reward on a single hop and $q = (1 - p)$, then the expected number of giving up hops for bouts of $5 = qp + 2q^2p + 3q^3p + 4q^4p + 5q^5p$. The observed points lie very slightly, but consistently above the random departure line, indicating that the birds tend to leave a perch after a run of bad luck. The observed points are $\pm$ standard error.

Treatment of results

The results of these experiments showed that the birds followed the general pattern of behaviour assumed by our version of the two-armed bandit model. There was an initial sampling period during which the birds performed on average 42% of hops on the more profitable and 58% on the less profitable perch. This proportion does not differ significantly from 50:50, and is consistent with the constraint of equal sampling in our model. The slight bias towards the less profitable perch resulted from our experimental procedure of always starting with the higher reward rate in whichever perch was used less in the preceding neutralisation test. After the decision point, the birds spent virtually all their time on the more profitable perch; on average, 95% of hops were on this perch, and the range for individual treatment means was 88–99%. Similar results, referred to as 'maximising' on concurrent variable ratio schedules, have been obtained with pigeons¹³.

In terms of our optimal decision model, we can consider a hop as being equivalent to a single trial, so that we can predict, for each combination of reward rates, how many hops on each perch the bird should do before making the decision to exploit. Figure 2a shows the observed mean decision point in each experimental treatment and the predictions of the model for three values of N (total number of hops in the experiment), 250, 150 and 50. The fit to the model with $N = 150$ is good; not only is the observed curve the same shape as predicted, but the predicted values are all fairly close to the observed means. The other values of N give curves of the same general shape, but with predicted values too high or too low. The value of N which fits the data (150) is the modal value for the number of hops performed in a test (Fig. 1b). This suggests that birds are optimising over the 'time horizon' which they experienced most often during our experiments, but we do not know if the birds would optimise with a bigger value of N if they were accustomed to longer tests. We plan to test this possibility. There is a slight difference in the modal value of test lengths for different treatments, but as the treatments were presented in a random order there is no reason to suspect that the birds could modify their behaviour according to treatment.

One way in which the birds' behaviour differed from that implied in our model is that they did not sample by doing one hop on each perch at a time, but instead they hopped in bouts of about five. (The overall mean bout length before decision was

5.00, the range 3.00–16.5.) This makes no difference in predicting the average decision point, but if the birds had hopped in rigidly determined bouts, the predicted decision point for a particular experiment would have to be a multiple of 5, which would involve reading every fifth matrix of the model's output. This tendency to hop in bouts probably explains why the observed points are above those predicted at the right-hand end of the graph where the bird should make a decision after about 10 hops.

The alternative goal we considered above was the strategy of immediate maximising, in which the bird always switches to the perch with the higher current expected reward rate. We used the β -distribution and the same starting values of α and β as above to predict, for any given experiment, at which point the birds should no longer switch back and forth if they were following this strategy. This predicted point is reached when expected payoff in the current patch never drops below that expected in the other one. The observed point was based on the decision criterion discussed above. The expected number of switches depends on the actual sequence of obtaining rewards in the first few moments of the experiment. In calculating the expected values we made the conservative assumption that the birds would continue to switch until the difference between patches in expected reward rate was greater than 5%. The observed mean values for the five treatments (50:5, 45:5, 40:10, 35:15, 30:20) were 3.3, 3.6, 3.8, 6.4 and 9.6, whereas the corresponding expected values were 0.93, 1.3, 1.36, 1.3 and 3.25. If we relax the assumption that the birds cannot discriminate differences of less than 5% in our experiments, the difference between observed and predicted values becomes even greater. Clearly, the birds switch more than expected. In other words, they are not maximising in the very short term, but instead they behave as if they acquire information to achieve a longer-term optimum.

Although the birds do not seem to switch according to the rule of immediate maximising, there is some evidence that switches between perches are related to short-term changes in reward rate. In Fig. 3 we plot the number of unsuccessful hops before a switch during the sampling period as a function of reward rate. The points lie very close to, but slightly above, the random departure curve. This random curve takes into account the fact that the birds work in bouts rather than single hops. Eight out of 10 means lie just above the random curve ($P \approx 0.05$). This shows that birds have a slight tendency to switch after a run of bad luck. In other words, they have a weak component of immediate maximising in their sampling strategy.

Discussion

The bird's behaviour approximates the prediction of a simple model based on a constrained optimal balance between exploration and exploitation, and does not fit the model of pure exploitation. The fit to the former model is close in spite of the fact that we have not yet directly incorporated a cost of switching between patches. In effect, we incorporated a small cost by using the rule that if the payoffs for sampling and exploiting were exactly equal, then the bird should exploit. In our experiment, this cost was small both in energetic terms and in time. However, in further developments of the model the cost of switching might be an important consideration.

Our comparison of the two maximising models has been in terms of predicting the details of the bird's behaviour, but an alternative approach is to compare the payoff, measured as number of rewards per test. On average, the two-armed bandit model achieves a slightly higher payoff than immediate maximising, except in very short experiments with less than five trials¹⁴, but when the reward rates are very different in the two patches (for example, 50:0) and the risk of a wrong decision is negligible, immediate maximising is just as effective. The difference between the birds' behaviour and that predicted by the two-armed bandit model, although small, is sufficient for the birds to do slightly worse than either of the two models in terms of payoff. However, the difference is less than 5% of the

predicted optimum of the two-armed bandit model (A.K. and J.K., in preparation). Thus, an analysis of payoffs enables us to say that the birds are close to the optimum but does not discriminate between the two models, unlike the analysis of the birds' behaviour.

We have presented averaged data for the nine birds we tested, but individual variation was very marked. We considered the possibility that some individuals are more cautious (do more exploring) but are less likely to make mistakes. Five of the nine individuals tested made one or more mistakes (that is, chose the poorer patch) in the 30:20 or 35:15 tests. These individuals did not make decisions after fewer hops than the other four birds. The average number of hops before a decision for all treatments was 23.9 ± 9.6 and 18.4 ± 9.8 for the fallible and infallible individuals, respectively. If, however, we examine individual treatments in which a bird made an incorrect decision, it is apparent that these tend to be cases in which the bird did little or no sampling. Examining the 18:20:30 treatments (nine birds, two treatments each), only three out of the 13 in which a correct decision was made involved no sampling, whereas two out of the five in which the bird made a mistake were tests in which there was no sampling period at the beginning.

Although the birds in our experiments behaved on average as if they were calculating an optimal balance between exploration and exploitation, we suggest that they use some simple rule to

approximate the optimum. We have simulated various potential rules including 'matching' future responses to rewards obtained so far, an empirically derived rule applying concurrent choice experiments¹⁵. The extent to which these proximate rules mimic the behaviour of the birds and match the total payoff achieved will be discussed elsewhere (A.K. and J.K., in preparation).

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Electrophoretic movement and localisation of acetylcholine receptors in the embryonic muscle cell membrane

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A steady electric field of 30 mV across a single embryonic muscle cell produces accumulation of acetylcholine receptors toward one pole of the cell within 1 h. The movement is electrophoretic in nature and the accumulation results in the formation of stable, metabolically independent receptor aggregates.

MANY cell membranes are fluid in structure¹, and proteins and lipids are free to undergo long-range movement in the plane of the membrane²⁻⁵. Under many circumstances, however, mobile membrane components may be localised and concentrated at specific regions of the cell membrane. The mechanism that modifies and stabilises their topographic distribution in the fluid matrix is poorly understood. One notable example is the acetylcholine (ACh) receptors at the neuromuscular junction. Embryonic muscle membrane before innervation is covered by ACh over the entire surface of the muscle^{6,7}. After innervation, the ACh sensitive site is essentially confined to the junctional region of the muscle membrane^{6,8,9}. Since muscle cell membranes are fluid^{10,11}, and redistribution of receptors during the course of innervation has been demonstrated^{12,13}. We present here our study of the induced movement and localisation of the ACh receptors in muscle cell membranes. We found that when a steady electric field of 10 V cm^{-1} (corresponding to a potential of 30 mV across a cell $30 \mu\text{m}$ in diameter) was applied to the surface of spherical *Xenopus* muscle cells, the receptors rapidly accumulated towards one pole of the cell. This accumulation of receptors is consistent with the electrophoretic redistribution of mobile, charged receptors in the plane of the cell membrane. The redistribution is independent of metabolic energy, blocked by the preincubation of the cells with concanavalin A (Con A) and reversed in direction after surface charge modification with neuraminidase. Moreover, the formation of the ACh receptors leads to the immediate formation of stable receptor aggregates which persist against diffusion, resist treatment with cytoskeletal disrupting agents and require no metabolic energy supply for their stability.

Electrophoretic mapping of ACh receptors

Single embryonic muscle cells, obtained by dissociating neurones of *Xenopus laevis* embryos (stage 18-19, ref. 1) were used as a monolayer on clean glass culture chambers and re-used for experiments after 2.5-3.5 d in culture. Culture medium contained 85% Steinberg's saline¹⁵ (58 mM NaCl, 0.0% Leibovitz medium (L-15, Gibco), and 5% fetal calf serum (Gibco). Within 2.5 d of culture, embryonic muscle cells of two distinct morphologies were observed, extended and rounded. All experiments were carried out on the extended mononuclear cells (diameter $35 \pm 5 \mu\text{m}$) that adhered tightly to the culture substratum (Fig. 1a). Details of the methods and apparatus have been reported elsewhere¹¹. Briefly, an electric current was applied to the thin, rectangular culture chamber of defined geometry ($6.0 \times 1.0 \times 0.2 \text{ cm}$) containing culture medium. A current of 1.5 mA

averaged $6.3 \pm 1.0 \text{ V nC}^{-1}$ (row 2, Table 1). Figure 1c shows representative asymmetrical responses of a cell after 1.5 h exposure to the field, and Fig. 2 plots peak depolarisation against charge ejected through the ACh pipette for two cells from the same culture. The sensitivities on the two poles of these cells were mapped before (cell a) and after (cell b) exposure to the field for 1.5 h. For the cell exposed to the field, the slope of the curve increased at the cathodal pole, and decreased at the anodal pole. In several experiments, we also mapped the sensitivity along the perimeter of the cell from 0° to 180° . It was consistently found that the positions with increased sensitivity were always centred around the 0° pole, whereas the sensitivities of all other positions decreased slightly from the control level. Figure 3 shows perimeter mappings for a control cell and three cells from three separate cultures exposed to the standard 10 V cm^{-1} field for 45 min, 1.5 h and 3 h, respectively.

To evaluate the degree of asymmetry in ACh sensitivity induced by the electric field, we defined an asymmetry index with the formula described in Table 1. This formula gives an index of 0 for a cell showing equal sensitivities on the cathodal and anodal pole of the cell, an index of 1 when the sensitivity is entirely confined to the cathodal pole, and an index of 0.5 when the sensitivity is threefold higher at 0° than that at 180° . By plotting mapping data for a series of cultures exposed to different durations of the same field, we found that the redistribution of ACh sensitivity reached a plateau asymmetry index of about 0.5 (Fig. 4).

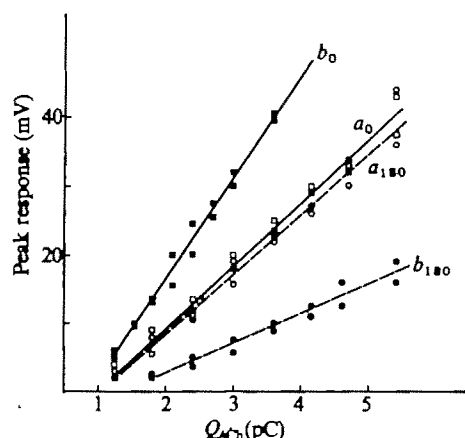


Fig. 2 Dose-response curve of the muscle cell membrane to applied ACh. Curves were obtained by plotting peak depolarisation produced by the picocoulombs of charge ejected from the ACh pipette. Slopes of lines drawn by eye were used as a measurement of sensitivity. Cells a and b were from the same culture and were mapped with the same ACh pipette before (a) and after (b) exposure to a field of 10 V cm^{-1} for 1.5 h. Sensitivities at 0° and 180° were 9.3 and 8.8 V nC^{-1} , respectively, for cell a, and in contrast, 14.5 and 4.3 V nC^{-1} , respectively for cell b. E_m during mapping ranged between -100 to -104 and -94 to -98 mV for a and b, respectively.

The asymmetric redistribution of ACh sensitivity was not due to the redistribution of acetylcholinesterase, the hydrolysing enzyme for ACh, over the cell surface. When iontophoretic mapping was carried out using a pipette filled with 3 M carbachol (Sigma), a non-hydrolysable ACh analogue, similar asymmetrical redistribution of sensitivity was found (row 4, Table 1). It is interesting, however that the absolute sensitivity of these cells towards carbachol is about fivefold lower than that towards ACh in identical iontophoretic conditions.

The redistribution of ACh sensitivity was a process independent of cell metabolism. In three separate experiments, cultures were preincubated in saline containing 10^{-2} M sodium azide, 10^{-3} M dinitrophenol and 10^{-3} M sodium fluoride for 45 min. This treatment blocks the contraction response of these muscle cells to bath-applied ACh (1 mM), indicating a

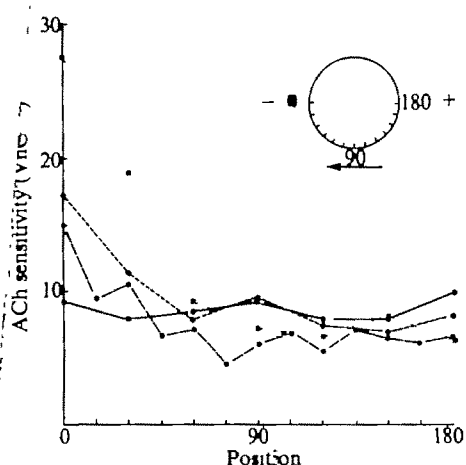


Fig. 3 Mapping of ACh sensitivity at various points along the perimeter of spherical muscle cells (inset). Four cells from four different cultures were exposed to a field of 10 V cm^{-1} for 0 min (●), 45 min (○), 1.5 h (◇) and 3 h (□). Arrow indicated direction of field.

depletion of metabolic energy. Cells so treated were then exposed to the standard field for 1.5 h, still in the presence of the metabolic inhibitors. Post-field mapping indicated that although the absolute sensitivities decreased on both sides of the cells (due to progressive loss of the membrane potentials), similar redistribution of ACh sensitivity still occurred (compare rows 2 and 5, Table 1). Consistent with the finding that the observed redistribution is a passive process driven by the external field, we found that cytoskeletal elements do not seem to be involved in the redistribution process. Preincubation of the cultures in saline containing cytochalasin B (Sigma, $10 \mu\text{g ml}^{-1}$) and colchicine (Sigma, $20 \mu\text{M}$) for 1 h does not affect the redistribution of ACh sensitivity subsequently induced by the field (row 6, Table 1). Such treatment is known to block active capping processes of surface receptors in other cell types²⁰.

The effect of Con A and neuraminidase

The above observations indicated that the redistribution of ACh sensitivity is consistent with a passive electrophoretic redistribution of ACh receptors in the plane of the cell membrane. If there was indeed a redistribution of the pre-existing ACh receptors, immobilisation of ACh receptors

Fig. 4 Rise of asymmetry index with increased duration in an electric field (10 V cm^{-1}). Data were from many experiments grouped for mappings done within 4 h of removal from field. Error bars represent 95% confidence levels. The figures indicate the number of cells mapped for each duration. Data with dashed error bars indicate cultures which were preincubated with Con A for 10 min before the field was applied.

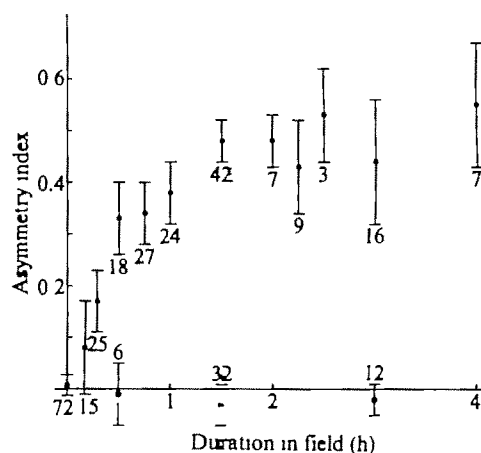


Table 1 Redistribution of ACh sensitivity induced by electric field

Duration of field exposure (10 V cm ⁻¹)	No. of cells mapped*	ACh sensitivity† (V nC ⁻¹)		Asymmetry index‡
		At 0° (cathodal pole)	At 180° (anodal pole)	
1. C (no treatment)	7 (11)	9.45 ± 0.65	9.44 ± 0.63	0.01 ± 0.02
2. 1.5 h	4 (5)	18.9 ± 2.3	6.3 ± 1.0	0.48 ± 0.04
3. 30 min	18 (3)	18.4 ± 3.8	9.1 ± 1.5	0.33 ± 0.07
4. 1.5 h (carbachol pipette)	17 (1)	4.0 ± 0.9	1.3 ± 0.4	0.49 ± 0.08
5. 1.5 h (metabolic inhibition)	14 (3)	15.6 ± 5.0	6.4 ± 1.5	0.37 ± 0.11
6. 1.5 h (treatment with cytochalasin B and colchicine)	15 (1)	10.6 ± 2.0	4.2 ± 0.6	0.42 ± 0.06
7. 1.5 h (preincubation with Con A)	19 (2)	7.3 ± 1.4	7.9 ± 1.2	-0.04 ± 0.08
8. 1.5 h (preincubation with neuraminidase)	22 (3)	6.5 ± 1.5	14.1 ± 2.4	-0.37 ± 0.08

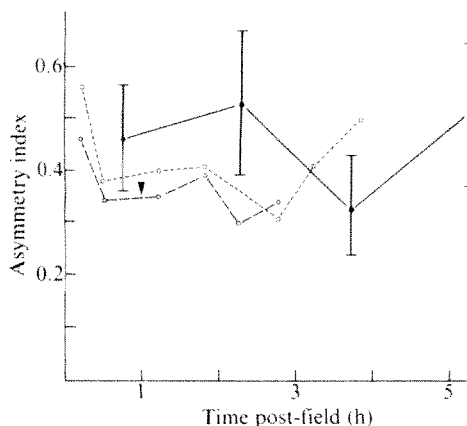
*All isolated spherical cells mapped successfully (membrane potential $|E_m| \geq -80$ mV, $\Delta E_m \leq 10$ mV during mapping) were included. Data were pooled from separate cultures (number of cultures shown in parentheses). As there was no relaxation of the asymmetry after the removal of field (see last section and Fig. 5), data were pooled regardless of the exact time the mapping was carried out.

†Sensitivity presented as (average) ± 95% c.l.

‡Asymmetry index = Sensitivity at 0° - Sensitivity at 180° / Sensitivity at 0° + Sensitivity at 180°, was calculated for each cell before averaging, and presented as (average) ± 95% c.l.

before the application of the field should prevent the redistribution of ACh sensitivity. The following experiments using Con A provide such a control. Con A is a tetravalent lectin molecule that specifically binds to the α -D-mannose or α -D-glucose residues of cell surface glycoproteins and glycolipids², and is known to crosslink and immobilise surface receptors². In control cultures not exposed to the field, incubation with Con A (Sigma, grade III, 150 μ g ml⁻¹) for 10 min did not greatly affect the absolute ACh sensitivity of the muscle cells. The distribution of ACh sensitivity was essentially uniform along the whole perimeter of the cells. For the cells preincubated with Con A then exposed to the standard field for various times, no significant asymmetry in the sensitivity was produced at the 0° and 180° poles (Fig. 4, open circles, and row 7, Table 1). We have shown previously¹¹ that preincubation with Con A blocks the electrophoretic accumulation of the Con A receptors, as indicated by post-field fluorescent Con A ring staining. As the ACh receptor is probably a subset of Con A receptors²², the present finding is consistent with the idea that immobilisation through Con A binding prevents electrophoretic movement of the ACh receptors.

Fig. 5 Stability of asymmetrical ACh sensitivity after removal from the electric field. Data from 10 separate cultures were grouped into 90-min bins and averaged for cells exposed to the field for 1.5 h without further pharmacological treatment (●—●). Data were grouped into 30-min bins and averaged for cells either exposed to the field for 1.5 h, and then treated with sodium azide and DNP (at arrow) for the remainder of the experiment (○—○) or treated with cytochalasin B and colchicine (□—□) during the entire experiment (1 h preincubation, 1.5 h field, and post-field mapping). Each data point, on average, represents seven independent measurements. For clarity, error bars (±95% c.l.) are included only for the 90-min bins.



The surprising effect of neuraminidase treatment on the redistribution of ACh sensitivity provided additional evidence that the redistribution is electrophoretic in nature. Neuraminidase removes sialic acid from the cell surface, reducing the negative charges on membrane constituents²³. We preincubated our culture with neuraminidase (Sigma, grade VI, 0.1 unit ml⁻¹) for 1 h at pH 6.6. The enzyme-containing saline was then substituted with culture medium and the field of 10 V cm⁻¹ was applied. Post-field mapping showed that the redistribution of ACh sensitivity reversed its polarity for all cells mapped (22 cells in three separate experiments; row 8, Table 1), as compared to cells not treated with neuraminidase. The sensitivity at the anodal pole increased greatly whereas that at the cathodal pole decreased. The reversal in the direction of redistribution is probably due to the modification of charged groups on the cell surface by the enzyme. The fact that ACh sensitivity increased on the cathodal side of the cells not treated with neuraminidase suggests that ACh receptors are positively or less negatively charged than other mobile membrane components. It is likely that ACh receptors are, in fact, negatively charged but contain fewer sialic acid moieties than other more negatively charged components. After neuraminidase treatment, they become among the most negatively charged components, and hence, accumulate at the anodal pole. It should be noted, however, that a full understanding of the electrophoretic redistribution of various membrane components still requires more information on the interaction between the charged groups on the cell surface and on the effect of various counter-ions on the charged moieties.

Stability of receptor aggregates

An interesting finding in the present study is that field-induced asymmetrical ACh sensitivity always persists after removal of the field. Figure 5 shows the asymmetry indices for cells mapped at various times after removal from a 1.5 h standard field. Previously¹¹, it was shown that field-induced accumulation of the most mobile population of Con A receptors in these muscle cells undergoes relaxation after removal from the field. Rapid back diffusion results in the recovery of uniform distribution within 30 min. In our present study, for cultures exposed to a field of 10 V cm⁻¹ for 1.5 h, the asymmetry indices persisted for as long as the mapping was carried out (up to 5 h). The stability of the field-induced receptor aggregates is not dependent on cell metabolism. Figure 5 (open circles) shows a series of mapping data obtained from cultures exposed to the field for 1.5 h, but in which the regular saline was replaced (at arrow) with saline containing metabolic inhibitors (10⁻² M sodium azide, 10⁻³ M dinitrophenol). For as long as the cells remained viable under this treatment, we found no significant change in the stability of the asymmetry. Drugs disrupting cytoskeletal structure also produced no effect on the stability.

Asymmetry persisted for up to 4 h in the presence of $10 \mu\text{g ml}^{-1}$ of cytochalasin B and $20 \mu\text{M}$ of colchicine in the saline (Fig. 5, open squares).

Although stable against back diffusion, the field-induced ACh receptor aggregates were still electrophoretically mobile. When a field of the same strength, but different polarity was applied to a culture previously exposed to the standard field, a second accumulation of the ACh receptors occurred at the new cathodal poles of the cells. The sensitivity at the original cathodal pole declined to the control level (unpublished data). Our observations on the accumulation of ACh receptors by the field, the stability of aggregates against back diffusion and their mobility under subsequently imposed fields of different polarity have now all been confirmed using fluorescently labelled α -bungarotoxin²⁴, a venom protein known to bind specifically to ACh receptors.

In conclusion, we have demonstrated the electrophoretic movement and localisation of a specific membrane protein/ionic channel in the plane of the cell membrane. The technique of *in situ* electrophoresis reported here may prove useful in perturbing the receptor topography on the cell surface and in dissecting the dynamics of receptor interactions. Whether endogenous electric fields within developing tissue are involved in the localisation of cell surface receptors remains to be elucidated.

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letters to nature

Origin of pregalactic microwave background

THE photon density in the observed thermal (~ 2.7 K) microwave background is $n_{\gamma 0} \approx 400 \text{ cm}^{-3}$. This contrasts with the present mean baryon density in the Universe, n_{b0} , which is in the range 10^{-5} – 10^{-6} cm^{-3} . In the standard 'hot big bang' cosmology, the ratio n_{γ}/n_b is essentially constant during the expansion, its value being

$$\mathcal{S} = n_{\gamma}/n_b \approx 3.6 \times 10^7 (\Omega h^2)^{-1}. \quad (1)$$

h denotes the Hubble constant H_0 in units of $100 \text{ km s}^{-1} \text{ Mpc}^{-1}$, and $\Omega = \frac{8\pi}{3} G \rho H_0^{-2}$ is the usual density parameter. $\mathcal{S}$ is an undetermined free parameter of the 'primordial fireball': the standard 'hot big bang' theory as yet gives no reason that $\mathcal{S}$ should be $\sim 10^8$ rather than (say) $\sim 10^4$ or $\sim 10^{12}$. Its observed value could be more readily understood in a different theory that attributes the radiative energy and entropy production to calculable processes in a specified epoch. There have been various proposals along these lines¹⁻⁹, but none has found as much favour as the standard 'hot big bang' cosmology. This is because production of the radiation at a redshift z requires a mass-energy conversion efficiency of the order

$$\rho_{\gamma}/\rho_b \approx 2.5 \times 10^{-5} (1+z)(\Omega h^2)^{-1} \quad (2)$$

This is implausibly high if z is large enough ($\gg 10^3$) to permit thermalisation by H and He plasma alone⁷⁻⁹; on the other hand, if z corresponds to the epoch of galaxy formation (or later), adequate thermalisation at centimetre wavelengths requires implausibly efficient opacity³⁻⁵. Here a possible non-primordial origin of the microwave background is outlined that seems less contrived than other such schemes.

For reasons outlined below, the dominant form of mass in the present Universe could be 'dead' remnants of massive or supermassive stars that formed when $t \leq 10^6$ yr. The active bright phase of these objects would last $\leq 10^7$ yr (and would be

over long before galaxies assembled as identifiable units) but could have generated enough radiation to satisfy equation (2) at $z \geq 100$. The uncondensed gas could, even at this early epoch, have been contaminated by the products of nucleosynthesis: it could contain heavy elements, molecules and dust, and would be dense and opaque enough to thermalise this radiation at a few hundred degrees, yielding a present-day microwave background with the characteristics observed.

To motivate a fuller astrophysical discussion of the radiation production and thermalisation, a general argument is outlined first which shows why this type of model naturally 'predicts' a value for $\mathcal{S}$ of order 10^8 .

Associated with any luminous gravitating objects where electron-scattering provides the dominant opacity is a critical luminosity, the 'Eddington luminosity' L_E , for which radiation pressure balances gravity. A characteristic timescale for conversion of rest mass into radiation is then¹⁰

$$\frac{Mc^2}{L_E} = t_* = \frac{c\sigma_T}{4\pi Gm_p} \approx 4 \times 10^8 \text{ yr} \quad (3)$$

The lifetime of stars is related to this by

$$t = \epsilon \left(\frac{L}{L_E} \right)^{-1} t_* \quad (4)$$

ϵ being the efficiency of the energy source¹¹. For massive and supermassive stars $L \approx L_E$; and the expression (4) also gives the time taken for an accreting black hole of mass M to radiate ϵMc^2 .

Suppose that at some early time t , some fraction F of the content of the Universe turns into self-gravitating objects which thereafter each radiate at a rate YL_E until they have radiated away a fraction ϵ of their mass-energy. This will take a time $(\epsilon Y^{-1})t_*$. At that time (assumed $\gg t_*$), the radiation energy density ρ_{γ} will be $\frac{3}{8}\epsilon F\rho_b$, the factor $\frac{3}{8}$ arising from redshift effects. But ρ_b is related to t by $\rho_b = (6\pi Gt^2)^{-1}$ (for $(1+z) > \Omega^{-1}$). If the radiation were to be thermalised, its temperature T_{rad} would

be given by $aT_{\text{rad}}^4 = \rho_r c^2$. When the radiation constant a is written explicitly as $\pi^2 k^4 / 15 c^3 h^3$ this yields

$$\left(\frac{kT_{\text{rad}}}{m_p c^2}\right)^4 = \frac{18}{5} \left(\frac{Gm_p}{e^2}\right) \left(\frac{m_e}{m_p}\right)^4 \left(\frac{e^2}{hc}\right)^{-3} Y^2 \epsilon^{-1} F \quad (5)$$

and, as the photons have average energy $\sim 2.3kT$, $\mathcal{S} \approx [m_p c^2 / (2.3kT)] \rho_r / \rho_b$ which can be written

$$\mathcal{S} \approx \left(\frac{e^2}{Gm_p^2}\right)^{1/4} \left(\frac{m_p}{m_e}\right) \left(\frac{e^2}{hc}\right)^{3/4} Y^{-1/2} \epsilon^{5/4} F^{3/4} \quad (6)$$

This argument can be generalised to situations when Y and F are time-dependent. However, we will always find an expression for $\mathcal{S}$ which involves a fourth root of the ubiquitous 'large number' ($\sim 10^{40}$) multiplied by a set of model-dependent factors whose product is generally somewhat less than unity. It is thus not *ad hoc* that the class of non-primordial models discussed here yields background radiation with $\mathcal{S} \approx 10^8$; on the contrary, equation (6) displays an explicit reason that this should be expected.

Three specific astrophysical arguments pointing towards a phase of pregalactic star formation are: (1) data on their dimensions and spatial distribution suggest that galaxies have developed from density irregularities in the early Universe with a smooth spectrum of form $(\delta\rho/\rho) \propto M^{-\alpha}$ ($\frac{1}{3} \leq \alpha \leq \frac{2}{3}$) (refs 12–15). If this spectrum continued towards smaller scales, then gas clouds of $\leq 10^6 M_\odot$ would already have condensed out—collapsing (and possibly fragmenting) into star-like objects—at $t \leq 10^6$ yr. (This conclusion is insensitive to the Universe's thermal history at earlier times.)

(2) More than 80% of the material in the Universe seems to be 'dark mass' in galactic haloes and clusters of galaxies^{15–17}. This is consistent with the view^{18–20} that most matter was incorporated into one or more generations of O or B stars or supermassive objects which, after a 'brief' ($\sim 10^7$ yr) phase at luminosity $\sim L_E$, collapsed to form dark remnants. These remnants subsequently clustered gravitationally on progressively larger scales, and provided the potential wells in which the luminous content of galaxies eventually condensed¹⁵.

(3) Recent evidence that Fe has almost a solar abundance in the intergalactic medium^{21,22} suggests that pregalactic nucleosynthesis^{19,20} may have occurred.

In this hypothesis, several per cent of the rest mass could have been converted to radiation, through O or B stars with $L \approx L_E$ supernovae, recycling of debris through successive generations of exploding stars or supermassive objects, accretion of dense surrounding gas on to compact remnants, and so on. Thus, even when $t \leq 10^7$ yr ($z \geq 100$), ρ_r/ρ_b can be lifted to the value of equation (2), so that—given adequate thermalisation—it can provide the whole microwave background.

Nucleosynthesis could have supplied heavy elements by $t \cap 10^7$ yr (even if they are not produced 'primordially' in some non-standard 'big bang'). At $z \geq 100$ the mean densities and probable composition throughout the entire Universe resemble interstellar clouds in our present-day Galaxy: we may expect molecules, and carbon or silicate grains. The radiation densities and path lengths are, however, orders of magnitude larger, and if the radiation were thermalised its temperature would be several hundred degrees.

If thermalisation processes can produce a 2.7 K background that is thermal at a present wavelength λ_0 (and we know the background is close to a black body spectrum at least for $\lambda_0 \leq 30$ cm) then the pregalactic medium must have been opaque at wavelengths $\lambda_0(1+z)^{-1}$ where $z \geq 100$ and $T \geq 270$ K.

Three contributors to the opacity are dust, molecules and the ionised component of the medium.

First, dust: the effective absorption cross-section of a grain of radius r can be written $Q(\lambda)\sigma_G$, σ_G being the geometrical cross-section. When $\lambda \gg r$, generally $Q(\lambda) \ll 1$. In fact, for spherical grains one expects $Q(\lambda) \leq Q_{\text{max}}(\lambda) = 4\pi(r/\lambda)$. This means that if $Q(\lambda) = Q_{\text{max}}(\lambda)$ the column density of material needed to yield optical depth unity at a wavelength λ ($\gg r$) is

proportional to λ but independent of r : for grains with typical density $\sim 2 \text{ g cm}^{-3}$ it is $\sim 2/3(\lambda/1 \text{ cm}) \text{ g cm}^{-2}$. The characteristic column density of the Universe at redshift z is $\sim 0.14(1+z)^{3/2}\Omega^{1/2}h \text{ g cm}^{-2}$. To provide optical depth ≥ 1 at redshift z for radiation whose present wavelength is λ_0 the fraction of material in the form of grains must satisfy

$$\frac{\rho_{\text{grain}}}{\rho} \geq 5 \left(\frac{\lambda_0}{1 \text{ cm}}\right) (1+z)^{-5/2} \frac{Q_{\text{max}}(\lambda_0(1+z)^{-1})}{Q(\lambda_0(1+z)^{-1})} \Omega^{-1/2} h^{-1} \quad (7)$$

Q is higher at shorter wavelengths, so whenever equation (7) holds at centimetre wavelength any radiation emitted in the optical or ultraviolet (UV) band will be quickly transformed (perhaps by a sequence of absorptions and re-emissions) into softer radiation and adequately thermalised within the expansion timescale for the Universe.

Second, molecules: if some heavy elements are combined into simple molecules (H_2O , CO, CS, and so on) then the lines corresponding to the rotational transitions will be smeared into overlapping continua by the Hubble expansion. If the levels are in thermal equilibrium (and so populated in accordance with statistical weights when separated by energies $< kT$) the condition for a given molecule, with fractional abundance n_{mol}/n and dipole moment $\mu \text{ Db}$, to provide $\tau > 1$ at $\lambda_0(1+z)^{-1}$ is²⁴

$$n_{\text{mol}}/n \geq 5 \times 10^{-4} \mu^{-2} (1+z)^{-3/2} (\lambda_0/1 \text{ cm}) \quad (8)$$

Third, free-free absorption by the ionised component: the primary sources of the radiant energy may supply UV photons capable of ionising the pregalactic medium. If there is sufficient dust to contribute to thermalisation, it will have a very large optical depth to these photons (see ref. 7). The grains are therefore very efficient at converting the energy of one 'hard' photon into many softer ones. Even though this means that most of the radiation energy density will be in the IR rather than the UV, there may, nevertheless, be enough photo-ionisations to maintain n_e/n at a significant fraction. The high density of soft photons has the interesting consequence that the Compton cooling time $t_{\text{comp}} \approx 3 \times 10^{11} (\tau_{\text{rad}}/270 \text{ K})^{-4} \text{ s}$ may be less than the recombination time $t_{\text{rec}} \approx 2 \times 10^{11} n_e^{-1} (T/270 \text{ K})^{1/2} \text{ s}$. In these conditions, the electrons may have $T_e \approx T_{\text{rad}} (\ll 10^4 \text{ K})$ even when n_e/n is high. Free-free absorption, important only at long wavelengths, will then enhance the thermalisation rather than distorting the spectrum²⁵. The free-free optical depth exceeds unity for

$$\lambda_0 \geq 2 \times 10^2 h^{-3/2} (1+z)^{-1/2} \Omega^{-3/2} \left(\frac{n_e}{n}\right)^{-1} \left(\frac{\bar{n}_e^2}{n_e^2}\right)^{1/2} \times \left(\frac{T_e}{2.7(1+z)}\right)^{3/4} \text{ cm} \quad (9)$$

but the value of n_e is too model-dependent to permit any firm estimate of how important this process is.

One example will show that thermalisation is no great problem for this general scheme. Suppose that $\Omega = h = 1$, and that 0.5% of the mass energy has been converted into radiation when $t \approx 3 \times 10^6 \text{ yr}$ ($z \approx 200$). This satisfies equation (2), and corresponds to a radiation rate of order L_E , assuming that most of the mass is involved in the pregalactic phase of star formation. Thermalisation at $\lambda_0 < 30 \text{ cm}$ would be guaranteed even if only $2.5 \times 10^{-4} (Q_{\text{max}}/Q)$ of the material were incorporated in grains. The actual value of Q depends on grain properties, but small grains of amorphous silicates may have $Q \approx Q_{\text{max}}$ at all wavelengths up to $\sim 1.5 \text{ mm}$ (refs 26, 27) (the wavelength at $z = 200$ corresponding to 30 cm at the present epoch). There is thus no need to invoke grains of exotic shapes or constitutions^{3,4}. Equation (8) suggests that molecules may be important: one requires only 3×10^{-6} (by number) in the form of some molecule with $\mu \approx 1$. The considerations leading to equation (9) suggest that free electrons in the pregalactic medium would be cooled by Compton drag to kinetic temperatures of a few hundred degrees: this provides a favourable environment for molecule formation²⁸.

The detailed physics of the thermalisation (and the epoch when it occurs) are quite uncertain: all that can be argued here is

that the general scheme is plausible in order-of-magnitude terms. The following types of observation are, however, relevant. (1) Background spectrum: although the spectrum should be essentially thermal, deviations from the Planck law might arise if energy were injected at epochs when thermalisation was no longer fully effective at all wavelengths. Also, if some volatile contributor to the opacity with a spectral feature at some wavelength λ_1 were to condense out at a well-defined temperature T_1 , then the background spectrum might now display a feature at wavelength $\lambda_1(T_1/2.7\text{ K})$. (2) Small-scale isotropy: the effective source of the microwave photons reaching us would lie at $z \geq 100$, when they were emitted by a grain or molecule, or scattered by a free electron. The expected amplitude of the temperature anisotropies due to inhomogeneities would be similar to those predicted by the 'hot big bang' picture. An interesting difference in the present scheme, however, stems from the fact that various wavelength-dependent opacities (and not just electron scattering) are important: this means that the angular fluctuations observed at different wavelengths need not be correlated, as the location of the 'cosmic photosphere' depends on wavelength. (3) Galaxy formation and 'missing mass': any observations bearing on galaxy formation, the value of Ω , and the nature of the 'dark' mass in galactic haloes and clusters are relevant to the ideas discussed here.

In conclusion, there are astrophysical reasons for expecting a large radiative output from pregalactic events when $t \leq 10^7$ yr. This radiation could be thermalised, and would now be comparable in temperature to the observed microwave background. It is thus tempting to suppose that essentially all this background originated in this way rather than being a vestige of a hot 'primordial fireball'. The general argument leading to equation (6) then allows us to understand why the parameter $\mathcal{P}$ —whose value is unexplained by the 'hot big bang' theory—is $\sim 10^8$. The arguments pertaining to helium and deuterium are thus our only real clues to the entropy and dynamics at much earlier eras; and the options of non-zero lepton numbers or non-Friedmannian dynamics complicate this issue (as do the uncertainties about nucleosynthesis in the pregalactic objects themselves). The very early Universe thus seems a topic for conjecture rather than consensus while such obscurity veils the first 3 Myr.

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Venus' rotation and atmospheric tides

VENUS rotates with a period of 243.00 ± 0.04 d retrograde¹. The obliquity, or angle between the spin vector and orbit vector is $178 \pm 1^\circ$ (ref. 1). Both the long period and the near 180° obliquity suggest that the spin has evolved under the influence of tidal torques. Tides raised by the Sun in the body of Venus would de-spin the planet in $\sim 10^8$ yr if no other torques were acting². The final state would be one of synchronous rotation (one side always facing the Sun). So either we are living during the final stages of Venus' tidal evolution, an unlikely circumstance, or else Venus has already reached a stable equilibrium in which other influences balance the solar body tide. It is possible that the Earth has 'captured' Venus into a resonance in which the spin period is 243.165 d. However, for this resonance to be a stable equilibrium, either Venus must have a gravitational field that is ~ 10 times 'rougher' than the Earth's³, a possibility that is largely ruled out by direct observation³, or a third torque must balance the body torque due to the Sun. Tides in the atmosphere driven by periodic solar heating could supply the necessary third torque^{4–6}, but no quantitative theory has previously been published. Such a theory⁷ is presented here, in which we argue that the current rotation is a stable balance between atmospheric and solar body tides.

Because the atmosphere is thin compared with the radius, tidal torques on the atmosphere depend only on lateral variations of atmospheric column density, which is proportional to surface pressure. Although less energy is absorbed at the surface than in the clouds (100 W m^{-2} compared with 500 W m^{-2} at normal incidence)⁸, heating at the ground is the dominant source of surface pressure variations. This follows from the 25-fold decrease in solar diurnal period, from 117 d at the surface to 4 d in the clouds⁸, and the fact that surface pressure oscillations vary roughly as $(\text{period})^2 \times (\text{pressure})^{-1/2}$ at the level of absorption.

It is not difficult to show that the power absorbed by the ground is quickly exchanged with the lower atmosphere. A simple model, representative of more distributed forcing, treats this heating as being confined to a thin layer above the surface. Because of the rigid lower boundary condition, such heating does not excite significant tidal oscillations in the atmosphere above, and fluid parcels within the heated layer remain approximately at constant pressure. The rate of change of column density is therefore

$$\frac{d}{dt} \int_0^\infty \rho dz = - \int_0^{z_0} \frac{\rho}{T} \frac{dT}{dt} dz = - \frac{F}{c_p T_0} \quad (1)$$

where z_0 is the thickness of the heated layer, t is time, ρ is atmospheric density, T_0 is surface temperature, c_p is specific heat at constant pressure, and F is the heat flux (in W m^{-2}) absorbed by the layer. To simulate the finite residence time of a heat pulse in the lower atmosphere we replace d/dt by $d/dt + 1/\tau$ where τ is a thermal time constant associated with boundary layer processes and is in general a function of the forcing frequency.

Equation (1) is solved for the column density as a function of planetocentric latitude, longitude, and time, assuming the lower atmosphere rotates with the solid body. The heat flux F is expressed as a function of these variables by assuming $F = 0$ on the night side and $F = F_0 \cos \zeta$ on the day side, where ζ is the solar zenith angle. The column density is then multiplied by the gradient of the tidal potential, crossed with the planetary radius vector and integrated over the spherical surface to give the net torque on the atmosphere. In a steady state, this torque must be transmitted to the crust. The torque may be resolved into a component parallel to the spin axis, which affects the magnitude ω of the spin angular velocity, and a perpendicular component which affects the obliquity. Defining the angle β as

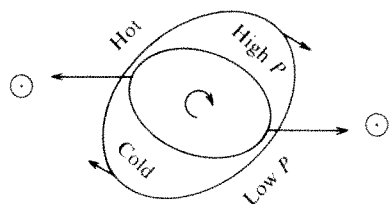


Fig. 1 Balance of torques on Venus. As only the semidiurnal components of the tides produce a torque, an 'image Sun' is shown diametrically opposite the true Sun, to make the picture symmetrical and to aid visualisation. The Sun is held fixed in this sketch so that Venus is rotating clockwise as seen from north of its orbit plane. The inner oval represents the tidally distorted figure of Venus, while the outer oval represents a surface of constant atmospheric pressure; the outer oval may also be regarded as the surface of an ocean of equivalent mass. Solar radiation raises the air temperature in the afternoon and causes mass to flow to the colder morning region. The high-pressure areas as well as the tidal bulges in the body of Venus are fixed with respect to the Sun (but not with respect to the surface). The gravitational attraction of the Sun, represented by arrows in the diagram, thus exerts contrary torques on the atmosphere and body of Venus.

the supplement of the obliquity, for $\beta \ll 1$ (as at present) one obtains

$$\frac{d\omega}{dt} = \left[\frac{3\pi n^2 r_0^4 F_0}{8c_p T_0 C} \right] \delta(2\omega + 2n) \quad (2)$$

$$\frac{1}{\beta} \frac{d\beta}{dt} = \left[\frac{3\pi n^2 r_0^4 F_0}{8c_p T_0 C \omega} \right] \frac{1}{2} [-\delta(2\omega + 2n) - \delta(\omega + 2n) + \delta(\omega)] \quad (3)$$

where

$$\delta(\sigma) = \sigma(\sigma^2 + \tau^{-2})^{-1} \quad (4)$$

Here n is the orbital angular speed, r_0 is the planetary radius, and C is the planet's greatest moment of inertia.

Equations (1)–(3) have a simple physical interpretation. Heating at the ground causes temperature to rise and column density to fall (equation (1)). Hence there is a mass concentration at the coldest time just after sunrise and a mass deficit at the hottest time just before sunset. The Sun's gravity acting on the sunrise side leads to a torque that tends to speed up the atmosphere (Fig. 1). The relevant frequency $2\omega + 2n$ is that of the solar semi-diurnal tide (equation (2)). The obliquity is affected first by the component of the semi-diurnal tidal torque that acts perpendicular to the spin axis (frequency $2\omega + 2n$), and second by the tides at frequencies ω and $\omega + 2n$ that result from the annual migration (at frequency n) of the sub-solar point across the equator, modulated (at frequency $\omega + n$) by the motion of the Sun relative to the surface (equation (3)).

The gravitationally induced tide that acts on the solid planet leads to expressions of form similar to those above²:

$$\frac{d\omega}{dt} = \left[\frac{3k_2 GM_\odot^2 r_0^5}{2a^6 C} \right] [-\epsilon(2\omega + 2n)] \quad (5)$$

$$\frac{1}{\beta} \frac{d\beta}{dt} = \left[\frac{3k_2 GM_\odot^2 r_0^5}{2a^6 C \omega} \right] \frac{1}{2} [\epsilon(2\omega + 2n) + \epsilon(\omega + 2n) - \epsilon(\omega)] \quad (6)$$

Symbols are defined in ref. 2.) The $\epsilon(\sigma) \approx Q^{-1}$ are phase lags with the same sign as σ that depend on dissipation in the solid planet. $Q(\sigma)$ is the quality factor for oscillations at frequency σ . Values of Q for Venus are unknown, but analogy with the Earth suggests $30 \leq Q \leq 100$ (ref. 9).

According to equations (2) and (5), atmospheric tides tend to increase ω away from synchronous rotation, while solar body tides tend to drive the spin toward synchronism. For $\tau \rightarrow \infty$ (long thermal response time), balance at the current diurnal period of 117 d requires $Q \approx 30$, as shown in Fig. 2. For smaller Q , balance at the current period requires larger Q . Equations (5) and (6) indicate that the current state is stable to obliquity

perturbations provided τ is less than about 2 weeks. The Earth resonance is also stable provided atmospheric and solar body tides balance at a period sufficiently close to the resonant period.

We have shown in which conditions the current rotation might be a stable equilibrium. As shown in Fig. 2, there is also a prograde equilibrium state with a diurnal period of 117 d (spin period ≈ 77 d). The stability of this state may be analysed by substituting $\pi - \beta$ for β and $-n$ for n in equations (2)–(6). Our analysis shows that when one of the two equilibrium states in Fig. 2 is stable to obliquity perturbations, the other is unstable.

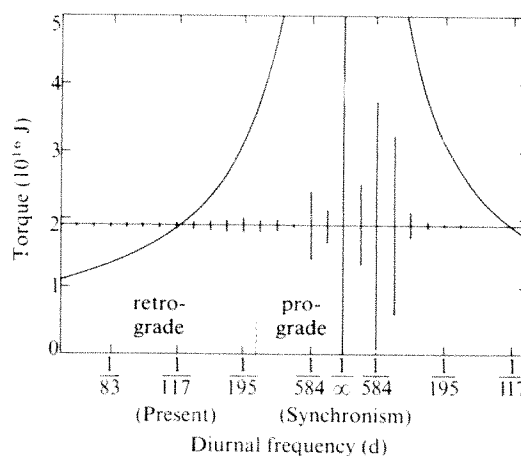


Fig. 2 Tidal torques plotted against the diurnal frequency $(\omega + n)(2\pi)^{-1}$ for spin vector parallel to the orbit vector. Atmospheric torques (curved lines) tend to drive the spin away from synchronism, and are shown for values of the thermal time constant $\tau \rightarrow \infty$. The solar tidal torque on the solid body (horizontal line) tends to drive the spin toward synchronism, and is shown for a value of the quality factor $Q = \text{constant} \approx 30$. This choice of Q ensures that atmospheric and solid body torques together drive the spin toward the current diurnal period of 117 d. Torques due to Earth resonances (vertical lines) tend to hold the spin period at the resonant frequencies, and are shown for a value of $(B - A)C^{-1} = 2.2 \times 10^{-5}$, similar to that for the Earth. Here A, B, C are the three principal moments of inertia of Venus, with $A < B < C$. For this amount of gravitational roughness, the Earth torques are too small by an order of magnitude to overcome the solar solid body torques, unless the solar torques and atmospheric torques balance near a resonant frequency. A recent analysis¹ of radar data suggests that the spin period is very close but not equal to that required for resonance with the Earth.

However, in general, other equilibria (both stable and unstable) can occur with obliquities between 0° and 180° . A more complete theory to be presented later indicates that Venus probably originated with a retrograde rotation in order to have evolved to the current retrograde state.

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Optical detection of local interstellar particles during an out-of-ecliptic mission

THE contribution to the zodiacal light (ZL) by interstellar grains has been estimated¹⁻³, but, as suggested here, new observations and analyses of the ZL and of the local interstellar medium provide a firmer basis for placing limits on the brightness of the interstellar (IS) relative to the interplanetary (IP) component. The proposed NASA/ESA 'out-of-ecliptic' (or 'solar polar') mission⁴ provides the best and perhaps the only observational method to distinguish between these two components.

To estimate the interstellar dust density, we make the usual assumption that the number density of the interstellar particles (n_{IS}) is related to the local hydrogen number density in the ratio of 10^{-12} (see ref. 5). The local hydrogen density within a few parsecs of the Sun has been observed to be $\sim 0.1 \text{ cm}^{-3}$ (ref. 6) which suggests that we are currently passing through an intercloud region of the galaxy. We infer from this gas density that the IS dust content is about $n_{IS} = 10^{-13} \text{ cm}^{-3}$.

The effect of the Solar System on the ambient IS complex of gas and dust is unknown. Although several mechanisms have been proposed either for capture^{7,8} or the sweeping out of interstellar grains, none of the results are conclusive because of uncertainties in the basic assumptions. The most efficient mechanism for deflecting interstellar particles (if charged) is probably the Lorentz force exerted by the magnetic field associated with the solar wind⁹. Levy and Jokipii¹⁰ estimate the ratio of the Lorentz to the gravitational force (F_L/F_G) at 1 AU to be 10, but their use of an IS particle size of $0.02 \mu\text{m}$ is too small by at least a factor of five for the classical size particles¹¹. If a realistic particle size of characteristic radius $0.12 \mu\text{m}$ is used, a value of F_L/F_G of the order of 0.4 (rather than >1) is derived, and sweeping out of the IS particles should not occur. Given the absence of detailed dust orbit calculations as well as the uncertainties in both the initial grain charge properties and in the possibility of the grains discharging in the IP medium, we base our calculations on the assumption that the grains are neither accreted nor swept out, but that the IS grains in the Solar System are uniformly distributed with a density characteristic of the local IS medium.

We estimate the monochromatic IS contribution to the ZL by scattering of solar radiation from an arbitrary distribution of interstellar particles from the brightness integral¹²

$$Z_{IS} = \int_{LOS} f_{\odot} \left(\frac{R_{\odot}}{R} \right)^2 n_{IS}(\theta) dl \quad (1a)$$

which becomes, for a uniform IS density,

$$Z_{IS} = n_{IS} f_{\odot} R_{\odot}^2 \int_{LOS} \frac{\sigma_{IS}(\theta)}{R^2} dl \quad (1b)$$

where LOS is line-of-sight, $f_{\odot}(R_{\odot}/R)^2$ is the solar flux at a typical volume element at a heliocentric distance R , θ is the scattering angle, $\sigma_{IS}(\theta)$ is the differential scattering cross section of the IS dust, and dl is measured along the LOS. The integral in equation (1b) is calculated from

$$\int_{LOS} \frac{\sigma_{IS}(\theta)}{R^2} dl = \frac{1}{b} \int_{\epsilon}^{\pi} \frac{F(\theta)}{k^2} d\theta \quad (2)$$

where b is the closest solar approach of the LOS ϵ is the elongation angle (see Fig. 1), and k is the wave number ($2\pi/\lambda$) of the incident radiation. For the scattering function $F(\theta)$, we use a dielectric dust model¹³. At a wavelength of $4,400 \text{ \AA}$, the conversion of cgs radiance units to the unit $S_{10}(V)$ (using the observational parameters recommended by Sparrow and Weinberg¹⁴) is

$$S_{10}(V) = 1.2 \times 10^{-9} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ sr}^{-1} \text{ \AA}^{-1} \quad (3)$$

where $S_{10}(V)$ is defined as the radiance produced by a 10th visual magnitude solar (G2V) star per square degree.

According to the model shown in Fig. 1, at the Earth ($b = 1 \text{ AU}$ and for $\epsilon = 90^\circ$), the IS contribution to the ZL is only about $1 S_{10}(V)$. This value is not only below the limit of detectability of $\sim 2-3 S_{10}(V)$ (ref. 15); it is also totally masked by the $200 S_{10}(V)$ contributed by the IP particles. On the other hand, viewing sunwards from a space craft (located at $R = 1 \text{ AU}$) along an LOS parallel to the ecliptic plane and $1/2 \text{ AU}$ above it (out-of-the-ecliptic), the IS brightness estimated from equation (2) for the then appropriate value of $\epsilon = 27^\circ$ is $\sim 28 S_{10}(V)$ which is well above the limit of detectability. Furthermore, this IS contribution may be distinguishable from the IP component.

An observational upper limit to the IS brightness component is established by noting that the ZL is close to solar colour from about $2,200 \text{ \AA}$ to $2.4 \mu\text{m}$ (ref. 16 and refs therein). Since the IS particle scattering is colour (wavelength) dependent in this range, this may be used to establish an upper limit to the IS component. Our calculations are confined to the wavelength spread of the UVB system where more data are available for separating the ZL and the background starlight. The ZL in this wavelength region is known to be solar colour to within about 5%.

Assume that the ZL consists of two components, the IP part which is solar colour and the IS component whose wavelength dependence follows the IS dust model previously cited. Thus if we consider the two wavelengths $5,400 \text{ \AA}$ and $3,600 \text{ \AA}$, the IP component has a spectral ratio $I_{IP}(5,400)/I_{IP}(3,600) = 1$ ($I_{IP} \neq I_{IP}(\lambda)$; I is normalised to solar colour). According to Greenberg and Hanner¹³, the IS component has a spectral ratio $I_{IS}(5,400)/I_{IS}(3,600) = 0.77$ at $\epsilon = 90^\circ$.

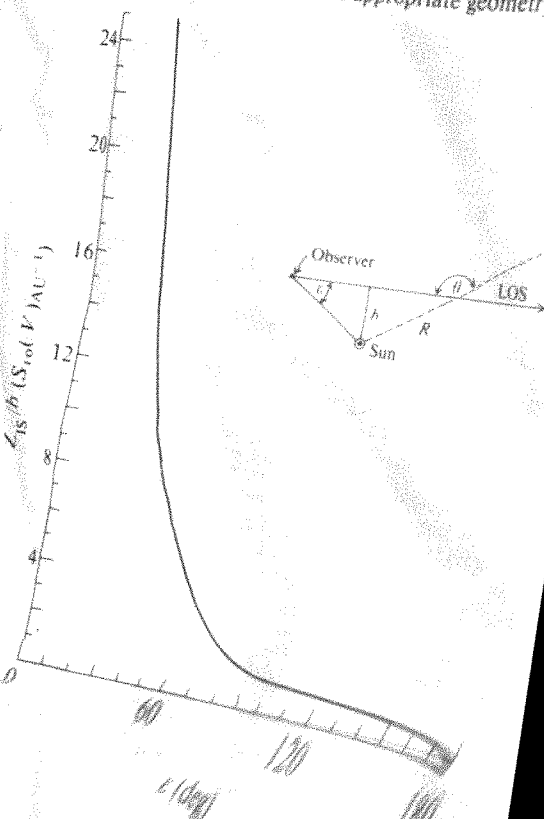
The 5% colour variation constraint then implies

$$\frac{I_{IP} + I_{IS}(3,600)}{I_{IP} + I_{IS}(5,400)} = \frac{1 + I_{IS}(3,600)/I_{IP}}{1 + 0.77 \times I_{IS}(3,600)/I_{IP}} \leq 1.05 \quad (4)$$

$$\text{so that } \frac{I_{IS}(3,600)}{I_{IP}} \leq 0.25 \quad \text{or} \quad \frac{I_{IS}}{I_{ZL}} \leq 0.20 \quad (5)$$

This means that no more than about 20% of the total zodiacal light as viewed in the ecliptic and at 90° elongation can be produced by scattering off of interstellar type particles.

Fig. 1 Resulting ZL brightness in $S_{10}(V)$ units per unit distance of closest solar approach along the line of sight from θ to $\theta = \pi$. Inset shows appropriate geometry.



As knowledge of the size and spatial distribution of the IP particles out of the ecliptic is lacking, we derive the maximum amount of IP dust $\frac{1}{2}$ AU off the ecliptic that would still allow the IS component to be distinguished. Let $f \cdot n_{IP}$ be this amount, where n_{IP} is the dust density in the neighbourhood of the Earth and f is the fraction to be determined. As a reference point, the neighbourhood of the Earth has a volumetric scattering cross section of $n_{IP} \sigma_{IP}(90^\circ) = 7.8 \times 10^{-23} \text{ cm}^{-1} \text{ sr}^{-1}$ (ref. 17). For $\theta = 45^\circ$, the scattering cross section $\sigma_{IP}(45^\circ)$ is about $1.77 \sigma_{IP}(90^\circ)$ (ref. 18).

Our minimum distinguishability criterion is that the ratio of the interstellar to interplanetary contributions be greater than 25%. For the case treated above for the interstellar contribution at $\frac{1}{2}$ AU above the ecliptic, then

$$\frac{Z_{IS}}{Z_{IP}} \approx \frac{n_{IS} \sigma_{IS}(45^\circ)}{n_{IP} \sigma_{IP}(45^\circ)} = \frac{7.5 \times 10^{-11} \times 10^{-13}}{f \times 1.77 \times 7.8 \times 10^{-23}} \geq 0.25$$

from which we obtain $f \leq 0.20$.

This value of f is close to but smaller than those derived from a series of models by Giese¹⁹ which produced results consistent with those inferred from observations from the Earth. Most of these models predict the dust density (assumed to be purely interplanetary) to decrease to 20% between heights of 0.5 and 1 AU above (or below) the ecliptic.

These rough calculations suggest there is a real possibility of optically detecting a local IS dust component in the Solar System. Although our criterion of distinguishability is based on colour arguments alone, both polarisation and scattering angle dependence provide additional tools to distinguish between the larger (IP) and smaller (IS) particles. The chance of isolating the IS component increases with decreasing elongation angle. Observations should be made along a line of sight which is parallel to the ecliptic and is as close to the Sun as possible. Unfortunately, such observations can also be inverted; not only the total brightness (Z) be obtained, but when the spacecraft near maximum height the value $n\sigma$ can be directly determined from the measurements²⁰.

Assuming that a positive identification of interstellar dust will be made, we are on the threshold of observing physical parameters of these particles which could previously only be inferred from observations of stellar extinction and scattering in interstellar nebula, which are normally hundreds of parsecs from the local neighbourhood. For example, given a slightly more optimistic estimate (by perhaps a factor of 2) of the local interstellar dust density, we might be able to observe how they are affected by interaction within the Solar System. S. acknowledges the support of NASA and the US Air Force Office of Scientific Research. We thank Dr R. H. Giese and Dr J. L. Weinberg for useful suggestions.

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Extent of hot intergalactic gas in the cluster Abell 2218

A SMALL cooling of the microwave background towards the centre of the rich, distant cluster of galaxies Abell 2218 has been detected¹⁻³. This decrement has been attributed to the inverse Compton scattering of photons of the background radiation by electrons in a hot intracluster plasma, as predicted by Sunyaev and Zel'dovich⁴. We report here new observations in several different directions towards the cluster which confirm the earlier results and provide an estimate of the extent of the gas.

The new measurements were made during the period February 1977 to May 1978 at the SRC Chilbolton Observatory, and consist of 199 h of observation at points north and south of the Abell cluster centre⁵ and 54 h further at that position. As in previous measurements^{1,3}, these observations were made at prime focus of the 25-m telescope operated at 10.6 GHz, using a twin-beam system with a beam-throw of 15 arc min in azimuth and HPBW of 4.5 arc min. The receiver incorporated a parametric amplifier with noise temperature about 180 K and bandwidth 250 MHz. The experimental method and data-reduction have been described in detail elsewhere³.

Table 1 shows the coordinates of the positions observed and the temperature decrements measured there. No corrections for the presence of nearby radio sources are needed⁶. The present results are consistent with earlier measurements near dec $66^\circ 20'$ —as discussed by Birkinshaw, Gull and Northover³. The measurement at dec $66^\circ 18'$ was made to check on the strong detections at $66^\circ 16'$ and $66^\circ 20'$ and to furnish another point close to the cluster centre. Its presence leads to an appreciable

Fig. 1 The galaxy strip count, N , in arc min bins and the observed temperature decrement at each point, ΔT , plotted as functions of declination, δ (1950.0 coordinates). The solid line is the ΔT profile of the best-fitting $\gamma = 5/3$, $\xi = -0.15$ atmosphere convolved with the beamshape of the Chilbolton telescope. The linear scale, D , at the distance of the cluster is also marked.

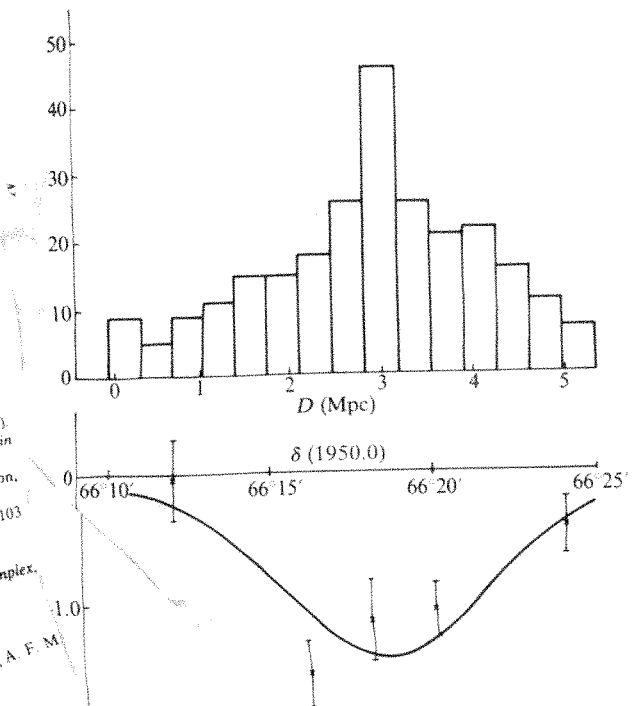


Table 1 Measured temperatures towards the observing positions in Abell 2218

Observing coordinates (1950.0)	Distance from cluster centre (arc min)	Time of observation (h)	Result (mK)	$\sqrt{\chi^2} - \sqrt{n-1}$
RA Dec				
16h 35min 42s 66° 12'	6.5	38	-0.03 ± 0.31	1.58
66 16	2.5	69	-1.49 ± 0.23	0.22
66 18	0.7	23	-1.11 ± 0.31	1.14
66 20	1.6	137	-1.05 ± 0.21	2.80
66 24	5.6	69	-0.42 ± 0.22	1.05

Table 2 Parameters of Abell 2218

Abell cluster centre (1950.0) ⁵	RA 16h 35min 42s Dec 66° 20'
New centre position (1950.0)	RA 16h 35min 37s ± 5s Dec 66° 18'.5 ± 0'.5
Best X-ray position (1950.0)*	RA 16h 35min 42s ± 40s Dec 66° 20' ± 4'
Distance class ⁵	6
Richness class ⁵	4
Redshift ⁷	0.207
Distance (for $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$)	1,262 Mpc
Cluster core radius, a (defined in text)	$(1.9 \pm 0.2) \times 10^{-4} \text{ rad}$ (240 kpc at A2218)
X-ray luminosity (2–10 keV)*	$(9 \pm 5) \times 10^{36} \text{ W sr}^{-1}$
Best-fitting atmosphere for $\gamma = 5/3$, $\xi = -0.15$;	
Central density	$2 \times 10^{-24} \text{ kg m}^{-3}$
Central temperature	$8 \times 10^8 \text{ K}$
Mass of gas	10^{45} kg
(Mass of gas)/(total mass of cluster)	0.08

* I. McHardy personal communication.

oversampling at the scan centre. In view of the asymmetry of the temperature decrements about the Abell centre at 66° 20', galaxy counts of this region were made from a copy of the Palomar Observatory–National Geographic sky survey red plate. New values for the position of the cluster centre and for the core radius, a , fitted to the assumed total density distribution $\rho(r) = \rho(0)(1 + r^2/a^2)^{-3/2}$ were determined from the counts—these and other cluster parameters are given in Table 2. Strip counts across A2218 and the measured values of the temperature decrement, ΔT , are plotted together in Fig. 1. It is evident that the Zel'dovich cooling is strongest at the cluster centre and decreases rapidly away from this point. A similar scan on a region of blank sky shows no such systematic deviations³.

To evaluate the reliability of the detection of an extended dip in the background, the data of Table 1 were fitted to four models; (1) no effect—a zero value at each point in the scan; (2) a constant effect, ΔT_0 , corresponding to a systematic offset; (3) a point-like 'source' at the cluster centre, parameterised by the peak cooling, ΔT_0 , and convolved with the beamshape; (4) an extended, adiabatic atmosphere with polytropic index $\gamma = 5/3$, and cluster filling factor³ $\xi = -0.15$, again convolved to the beam and parameterised by ΔT_0 .

In the last three cases, the best-fitting values for ΔT_0 were found, and χ^2 were calculated for all the models. The results were: (1) $\chi^2 = 83.4$, 5 d.f.; (2) $\chi^2 = 20.1$, 4 d.f.; (3) $\chi^2 = 13.7$, 4 d.f.; (4) $\chi^2 = 6.4$, 4 d.f. These values are such that all but the last hypothesis can be rejected at the 1% level (the probability of a greater value of χ^2 for model 4 is about 0.18). Hence our data constitute strong evidence for an extended atmosphere in Abell 2218. The ΔT values corresponding to the best atmosphere of type 4 are plotted in Fig. 1. The central temperature and density required to produce the observed X-ray flux density and temperature decrement are given in Table 2. The values of these parameters are uncertain by a factor ~ 3 due to the range of possible values of ξ .

Because the cooling effect, X-ray emission and galaxy distribution are strongly correlated in position, we conclude that the evidence for a microwave background decrement associated with a hot intracluster medium in Abell 2218 is now overwhelming.

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Limited solubility of iron in the Sun's interior

STRIPPED iron nuclei in a hydrogen plasma under central solar conditions, according to the classical Debye–Hückel model, would undergo phase separation for concentrations well below the cosmic abundance value. The higher concentration corrections, needed to characterise the iron-rich phase, lead to enhanced solubility for a simplified model where the electrons form a uniform background. Support for an iron-rich phase coalescing in the solar interior requires more accurate treatment of bound and partially bound electrons in such a mixture. The results of the Debye–Hückel model where the electrons are treated discretely and as a continuum, are reported here and support the possibility of phase separation. The physical cause of that phase separation is simply that the potential energy is lower in the separated phases than in the mixture because the local charge neutralisation is much better satisfied in the two separated phases.

Recent phase separation calculations for mixtures of stripped atoms^{1–3} suggest that highly charged nuclei would have limited solubility in a hydrogen plasma under conditions found in stellar interiors. These results suggest that highly ionised iron would precipitate out in the solar interior and coalesce in the centre. Should this occur and lead to a reduction of the iron concentration near the thermonuclear burn region, by an order of magnitude below the cosmic abundance value (2.5×10^{-5} ionic mole fraction), the existing neutrino dilemma might be resolved. It is estimated⁴ that such a concentration reduction would lower the opacity, and hence the temperature, sufficiently to significantly reduce the probability of fusion of α particles that generates the nuclei whose neutrino emission is being monitored. This paper presents various simplified models for the iron, hydrogen plasma phase diagram which explore this possibility.

A realistic calculation of the phase diagram is difficult for two reasons. The most important difficulty is that of accurately treating the bound and partially bound electrons of iron. At the solar interior temperature of about 1.5 keV and pressure of 10^5 Mbar, the two 1s electrons (with ionisation potentials of over 8 keV) are still bound whereas the eight 2s and 2p electrons (with ionisation potentials below 2 keV) are partly bound.

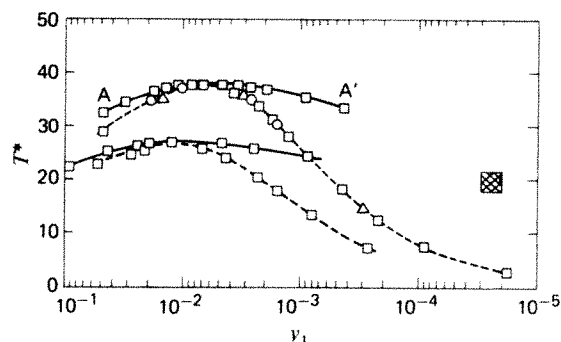


Fig. 1 The coexistence (solid lines) and instability (dashed lines) curves for an element of charge 26 (upper set of curves) and of charge 20 (lower set of curves) in a hydrogen plasma according to the Debye-Hückel theory. The ionic mole fraction of the element, y_1 , is plotted against the reduced temperature, T^* , at various pressures (Δ , 10^3 Mbar; $\square$, 5×10^4 Mbar; $\circ$, 10^4 Mbar). Points A and A' are, for example, two coexisting phases in the charge 26 system. The hatched area represents central solar conditions.

Of course, the bound-free transitions of these electrons lead to the large contribution to the opacity of a hydrogen plasma of even a very small amount of iron. This problem is crudely dealt with here by ignoring the partly bound electrons and considering the bound electrons merely to reduce the point nuclear charge to give some effective point charge. This crude approximation may not as seriously affect the calculation of the coexistence curve as might at first be expected. Errors tend to cancel as the chemical potential of iron in the iron-rich phase is equated to that in the iron-poor phase, where in both phases the electron density is nearly the same. In previous calculations³, when the polarisation of the electron gas by the ionic charge distribution was treated by perturbation theory the phase diagram was not drastically modified.

The second difficulty concerns accurate treatment of concentrated solutions of electrolytes, such as the iron-rich phase. The dilute phase can be adequately dealt with by the classical Debye-Hückel theory of a mixture of three components (iron nuclei with an effective charge, protons and electrons) as can be seen from the ratio of the ion sphere radius to the Debye length being less than unity (see Table 1). The electrons in the solar interior are very non-degenerate, as can be seen from the calculated Fermi temperature (Table 1). To avoid dealing with the concentrated solution, the instability curve was calculated in the dilute phase from the vanishing of the second derivative of the free energy with respect to iron concentration. This limit turned out, however, to be too loose to give insolubility near solar concentrations, so we had to deal with the iron-rich phase by corrections to the Debye-Hückel theory.

These corrections require a quantum mechanical treatment of the electrons to avoid divergences which arise if bound state contributions are ignored. These corrections can be included using an activity expansion⁵ but are of such complexity that they have not yet been calculated for this system. Instead, a model treating the electrons in the uniform background approximation was explored. Comparison with the discrete case discussed earlier, where in both cases the ions were treated in the Debye-Hückel approximation, was satisfactory, demonstrating the insensitivity of the coexistence curve to the electron distribution. For the uniform electron model it was then relatively easy to calculate the higher concentration⁶ corrections and show how they affect the coexistence curve. Even these corrections fail to extend the coexistence curve into the solar concentration region since the series fails to converge for the coexisting iron-rich phase.

Having exhausted so-called analytical methods, the uniform electron background model can be extended by numerical techniques using either the Monte Carlo or molecular dynamics method. These calculations are fairly cumbersome, so they will

be used initially to check how well the hypernetted chain integral equation represents the mixture of charged nuclei in a neutralising rigid uniform electron background. If that integral equation were also adequate when the charge difference between the species is extreme, as in the present case, it would reduce the numerical work required. The object of these calculations would be to determine the solubility for an iron ion of charge 24, corresponding to two bound K electrons, in a hydrogen plasma in which the polarisation of the partially bound L electrons will be treated by linear dielectric response theory.

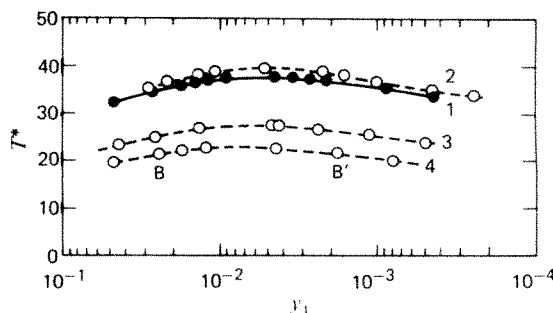
For a mixture of N_2 protons and N_2 ions of charge Z_1 and hence $N_1 Z_1 + N_2$ classical electrons in a volume Ω , the low density value of the free energy is given by the Debye-Hückel theory⁷, which requires that the ratio of the ion sphere radius r , to the Debye length, λ , to be < 1 .

The Debye length is given by $\lambda^{-2} = 4\pi\rho e^2 Z^2 / kT$, where $\rho = 3/4\pi r^3$ is the total particle density and defines the ion sphere radius. $Z^2 = X_1(Z_1^2 + Z_1) + 2X_2$, where X_1 is the concentration per particle or $X_1 = N_1/N$ with $N = 2N_2 + (1 + Z_1)N_1$. We define the concentration per ion as $y_1 = N_1/(N_1 + N_2)$.

The pressure and Gibbs free energy thus derived are used to investigate phase separation at constant temperature and pressure, by the double tangent construction which is equivalent to equating chemical potentials. The instability points determined by setting the second derivative of the Gibbs free energy, with respect to mole fraction, equal to zero, always lie within the concentration range determined from the double tangency construction. The instability curve for the low concentration phase can be calculated over a wider concentration range than the phase diagram, as shown in Fig. 1, because it is not limited by the validity of the Debye-Hückel approximation for the coexisting concentrated phase. However, the applicability of the Debye-Hückel theory as given by the r/λ criterion for the free energy is severely tested when a second derivative of the free energy is taken, indicating that r/λ must be $\ll 1$ before the second derivative is adequately given by the low density theory. But as shown in Fig. 1, the instability criterion is not restrictive enough. For phase separation to occur, solar conditions must lie in the two phase region, that is below the solid curves. If solar conditions are also below the dashed curves, the instability criterion would have been sufficient.

The coexistence curve and instability curve for $Z_1 = 26$ and $Z_1 = 20$ are displayed in Fig. 1 at various pressures. The results are nearly pressure independent when plotted as a function of the reduced temperature, $T^* = kT/e^2\rho^{1/3}$, because the equation of state is almost that of an ideal gas. This is indicated by the large value of T^* which is a measure of the ratio of kinetic

Fig. 2 The coexistence curves for an element of charge 26 in a hydrogen plasma according to the Debye-Hückel theory (curve 1), according to the Debye-Hückel theory for the ions but with the electrons treated in the uniform background approximation (curve 2), according to the same model as represented by curve 2 except that the first order concentration corrections as given by Abe are included (curve 3) and according to the previous model except that the second order concentration corrections are also included (curve 4). The ionic mole fraction y_1 , is plotted against the reduced temperature, T^* , at a pressure of 5×10^4 Mbar. Points B and B' are, for example, two coexisting phases.



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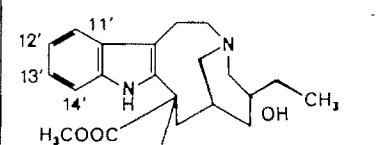
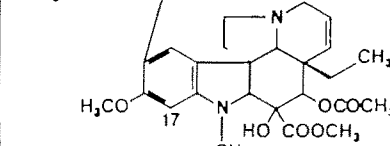
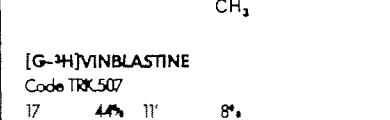
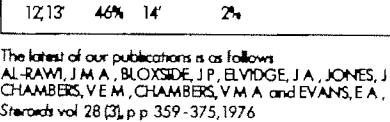
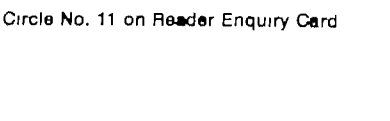
Distribution of labelling in tritium compounds

Modern techniques for the production of tritiated compounds are more sophisticated than those used in the early days of tritium labelling, and produce compounds labelled in specific positions rather than generally labelled. Nevertheless, it is necessary for many tracer applications of tritium compounds to know the *precise* position and configuration of the tritium labels. Traditional chemical methods of doing this are tedious and time consuming and subject to considerable error, and so the routine supply of such information has until recently not been possible.

The Radiochemical Centre, in collaboration with the University of Surrey, has developed over the past eight years the technique of tritium nuclear magnetic resonance (tnmr) spectroscopy for this purpose. This method is much quicker and more accurate than the traditional chemical or biochemical methods for determining distribution of tritium labelling.

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13'	14'	18	39.9%
		[6-3H]BENZO[a]PYRENE Code TRK 501	
17	17	6	<5%
		D-[2-3H]GLUCOSE Code TRK 361	
17	11'	2(c)	60%
12,13'	14'	2(b)	60%
		[G-3H]VINBLASTINE Code TRK 507	
17	11'	2	8%
12,13'	14'	3	28%
		L-[3,4(n)-3H]VALINE Code TRA/K 533	
2	4	4	64%
3			

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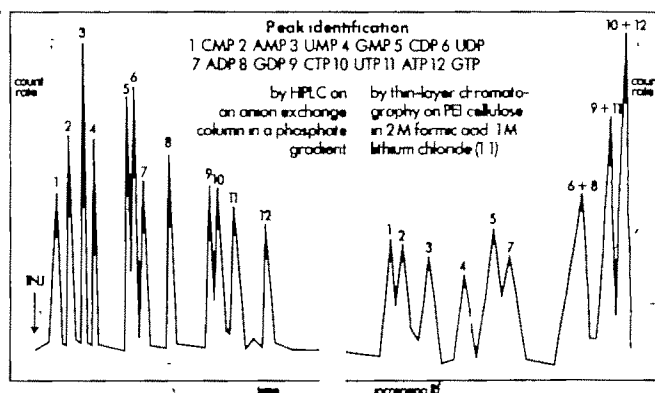
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[³H]G.I.628 TRK 595 15-30 Ci/mmol
[N-methyl-³H]Flunitrazepam
TRK 590 30-60 Ci/mmol
[G-³H]Piperidine hydrochloride
TRK 589 30-60 Ci/mmol

2-Deoxy-D-[U-¹⁴C]glucose
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Table 1 Conditions for coexisting phases* and for the solar centre† (a) and the corresponding convergence of the Abe expansion (b)

a					
y_1	$T(10^7 \text{ K})$	$V(\text{cm}^3 \text{ mol ion}^{-1})$	P	r/λ	T/T_F
1.8×10^{-3}	1.15	0.03924	50,000	0.62	4.3
2.5×10^{-2}	1.15	0.04805	50,000	1.71	3.6
2.5×10^{-5}	1.60	0.00902	300,000	0.46	2.3
b					
y_1	P_{el}	$P_{\text{DH}}^\ddagger$	$P_{\text{Abe 1}}$	$P_{\text{Abe 2}}$	
1.8×10^{-3}	25,857	24,051	77	15	
2.5×10^{-2}	32,969	14,398	2,002	631	
2.5×10^{-5}	153,224	146,737	38	1	

* The first two entries correspond to conditions B and B' in Fig. 2, respectively.

† The third entry.

‡ The Debye-Hückel pressure includes the ideal gas term, all pressures are measured in Mbar.

to potential energy. The solar conditions in Fig. 1 indicate that extending the coexistence curve would predict considerably smaller solubility than the cosmic abundance value of 2.5×10^{-5} in which the iron-rich phase would contain about half iron and half hydrogen. Even for the extreme case of $Z_1 = 20$, believed to represent the lowest possible ionisation state of iron under interior solar conditions, phase separation might be marginally inferred. The Debye-Hückel theory predicts, as expected, that decreasing the effective iron charge lowers the critical temperature and increases the critical concentration. The prediction is, therefore that in any stellar interior in which the difference in charge between iron and any other charged species is >20 , iron will be less soluble than its cosmic abundance. In the Sun the temperature drops at midradius sufficiently for iron ionisation to be reduced below 20 and hence beyond this midpoint, iron would be expected to be soluble.

Because the dilute Debye-Hückel theory is insufficient and the rigorous corrections require the electrons to be dealt with quantum mechanically, a uniform background electron gas approximation was explored for which concentration corrections can be more easily calculated. In that case, the free energies of the ions and electrons are additive. The electrons are treated as an ideal Fermi gas whose thermodynamic properties involve the usual Fermi-Dirac integrals⁸. For the temperature and pressure of interest here, the electron gas is nearly classical. The ions are first treated in the Debye-Hückel approximation, and the case for $Z_1 = 26$ is compared in Fig. 2 with the earlier calculation where the electrons were treated as an ionic species. The similar coexistence curve encourages the uniform background model to be extended to higher densities.

The straightforward inclusion of the first order correction^{5,6} leads to a rather large shift of the coexistence curve as shown in Fig. 2. Hence, it was necessary to consider the second order correction. The coexistence curve calculated including this correction to the free energy, is the lowest curve in Fig. 2 and shows that the shift in the critical temperature is considerably smaller than with the first correction. Extrapolating this series from these limited results leads to an estimated critical T^* , for the uniform electron gas case of about 20. This T^* is larger than that for central solar conditions, which is necessary for insolubility exceeding cosmic abundance.

Table 1 shows the convergence of the series for the pressure, at pressures corresponding to the region of thermonuclear burn and at the centre of the Sun. In the dilute iron phase, the electron and ion pressure are nearly equal, corresponding to ideal gas conditions of a hydrogen plasma, and the series convergence is excellent. In the coexisting iron-rich phase, the electron pressure exceeds the ion pressure due to the large number of electrons ionised from a high Z element. Furthermore, the convergence of the series is such that it is not possible to calculate the coexistence curve reliably for a more iron-rich

phase. Nevertheless, the calculations presented here suggest phase separation under solar conditions. More realistic models are needed to confirm this suggestion.

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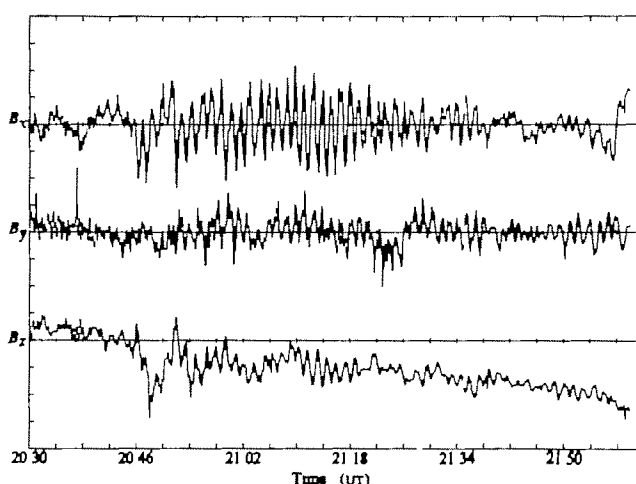
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Alfvén waves generated by an inverted plasma energy distribution

OBSERVATIONS from the geosynchronous spacecraft ATS6 of magnetic oscillations transverse to the Earth's magnetic field in the presence of a plasma ion distribution with a 'bump-in-tail' form are reported here. We argue that the oscillations are Alfvén waves, that the non-monotonicity (that is, population inversion) of the energy distribution provides a source of free energy for the oscillations, and that the particle-wave interaction is through bounce resonance¹. The data reported here comes from the UCLA fluxgate magnetometer² and the UCSD particle detector which measures ions and electrons with energies from 10 eV to 80 keV (ref. 3). The magnetometer data are displayed in a field aligned coordinate system in Fig. 1. A wave with an amplitude of about 5 nT and a period of about 100 s is present. The strongest signal is in B_x , the component in the meridian and perpendicular to the background field, B . The magnetic signal seems typical of a class of pulsations with short wavelengths across B seen in the local afternoon on ATS6⁴. These particular observations were made near 1500 LT.

Figure 2 shows the ion-velocity distribution, f , as a function of energy, W . The curves show the mean distribution for

Fig. 1 The magnetic field data from the geostationary spacecraft ATS6, are presented in a mean field aligned coordinate system in which B_x is measured along the mean field direction, obtained by forming a 10 min sliding average of the data. B_x is in the plane containing the Earth-satellite line and B_z . The B_z baseline is 130 nT. One division on the ordinate is 2 nT. Peak to peak amplitudes in B_x are of order 5 nT.



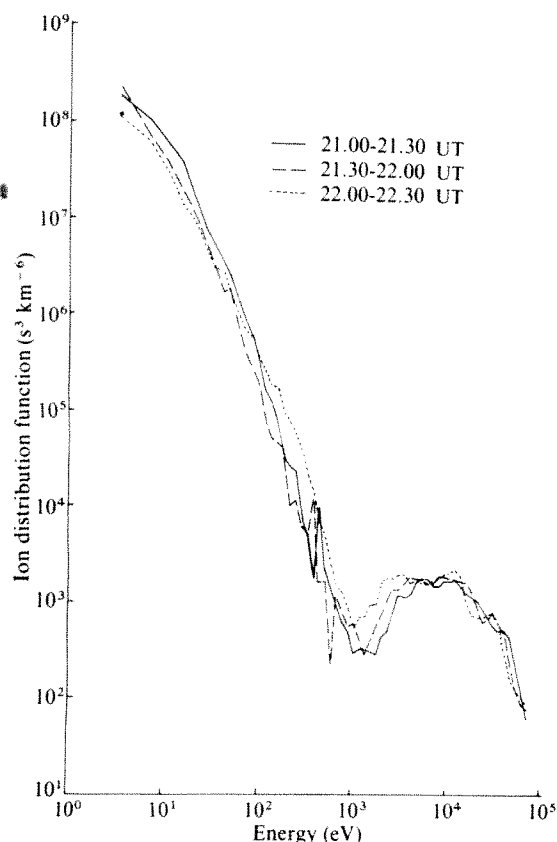


Fig. 2 Ion velocity distribution functions measured on ATS6 during the three half-hour periods during which the magnetic oscillations were observed. Although the instrument cannot distinguish ion species directly, we assume that the ions are protons.

three successive half-hour segments. The distribution remained largely unchanged through the course of the event. There is a minimum where $W \sim 1$ keV and $\partial f / \partial W > 0$ in the range $1 \text{ keV} < W < 10 \text{ keV}$. Any 'filling' of the distribution with time is probably due to spatial structure in the ion distribution, since the ions are drifting in the opposite sense to the spacecraft motion in this region⁵. In a collisionless plasma, diffusion in energy to fill the minimum may be accomplished by particles being resonantly scattered by a plasma wave. The observed frequency is of the order of the frequency of the bounce motion of an ion with a few keV energy in the Earth's mirror field and so the relevant resonant interaction is bounce resonance¹.

The low frequency and the quasi-transverse nature of the magnetic signal suggest that the wave present is an Alfvén wave with standing structure along the geomagnetic field. This idea is confirmed by more detailed investigation. The UCSD detector measures a complete energy spectrum every 24 s, and the effects of the wave can be detected in the low energy proton channels. The variation in low energy proton count is due to a drift velocity proportional to the wave electric field⁶ and using procedures similar to those used by Hughes *et al.*⁷ we found the wave electric field was in quadrature with the magnetic signal thus confirming the standing nature of the wave. The relative phase also showed that the standing wave was an even harmonic (a mode with an electric field node at the equator) and the observed frequency together with an estimate of the Alfvén speed, based on observed densities, suggests it should be the second harmonic. The symmetry of the signal about the equator is crucial in suggesting bounce resonance is the source; the first descriptions¹ of the mechanism pointed out that even harmonics would be generated. Other hydromagnetic wave sources are likely to generate the fundamental. The wavelength of the signal across the field is short, since magnetic pulsation activity on the geosynchronous SMS1 spacecraft (19° E of ATS6) and SMS2 (10° W of ATS6) did not correlate with the

ATS6 signal. This shows signals are relatively localised and rules out the Kelvin-Helmholtz instability as the energy source⁴. Spectra and coherence information from the three spacecraft for this event are shown in Fig. 4 of ref. 4.

The condition for a particle with bounce frequency, ω_b , and E-W drift speed $\tilde{\omega}_d$ to be in bounce resonance with a wave with frequency ω and E-W angular wave number m is

$$\omega - m\tilde{\omega}_d = N\omega_b$$

where N is an integer¹. Here we are interested in $N \sim 1$ and if the Doppler shift $m\tilde{\omega}_d$ is significant the E-W wavelength needs to be relatively small¹ (of the order of the equatorial Larmor radius of a resonant particle).

A particle's energy in an Alfvén wave increases due to the wave electric field at a rate $q\mathbf{E} \cdot \mathbf{v}_d$, where $\mathbf{v}_d$ is the E-W drift velocity due to curvature and ∇B drifts. We can estimate the energy diffusion coefficient due to this acceleration for a particle in resonance with a wave of bandwidth, $\Delta\omega$,

$$\langle q\mathbf{E} \cdot \mathbf{v}_d \rangle^2 \Delta\omega^{-1}$$

where the brackets denote the mean value seen over a particle bounce. We can estimate the wave electric field as $\sim 5 \times 10^{-1} \text{ mV m}^{-1}$ from the data and substitute this value into the diffusion coefficient to show that the time scale for diffusion to fill the hole in the energy distributions is $\sim 5 \times 10^5 \text{ s}$ that is a time much longer than the time scale of particle motions through this part of the magnetosphere.

The filling of the distribution seen in Fig. 2 cannot, therefore, be due to the waves. Cowley⁵ has shown how the magnetospheric convection pattern can lead to such distributions and, as indicated earlier, the protons are drifting in the opposite sense to the spacecraft motion. Much as one can estimate a diffusion time one can parametrise the energy exchange between particles and waves by estimating the contribution to the growth rate of the resonant ions. Estimates⁸ show that this is proportional to the resonant particle pressure:

$$\gamma/\omega \approx P_{\text{res}}/B^2/2\mu_0$$

which gives

$$\gamma/\omega \sim 10^{-2}, 10^{-3}$$

for observed parameters. These figures are of a similar order to the expected rates of ionospheric damping on the dayside of the magnetosphere⁹ and it seems the quasi-steady signal is maintained by energy being fed from particles to the wave to be lost in the ionosphere. The amplitude of the waves would then be sustained by the supply of ions drifting from the night side.

There are also low energy electrons in bounce resonance with the wave. Although there are more electrons than ions, their pressure is much lower as the resonance condition implies they have the same velocity as the ions.

In conclusion these observations show the first direct evidence of generation of hydromagnetic waves through bounce resonance with a non-equilibrium distribution, which theory first suggested 10 years ago^{1,10}. In this observation the distribution of ions was non-monotonic with energy. Theory shows that this is not a necessity; steep spatial gradients in the ion distribution can similarly drive waves. However, both observation¹¹ and theory⁵ indicate the regular occurrence of non-monotonic ion distributions in the afternoon-evening magnetosphere. These may be the source of many of the pc4 pulsations characteristic of this sector at geosynchronous orbit.

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A fast interferometric chopper for neutrons and X rays

FAST neutron choppers have been considerably developed in the past few years. The commonly used choppers are either rotating massive objects through which appropriately shaped neutron channels are cut¹ or crystals whose diffracting power may be modulated by magnetic fields² or dynamic strains³. All these devices suffer from practical limitations of speed, poor duty cycle and the consequent loss of intensity. We describe here our use of a scanning Bragg reflecting crystal interferometer as a fast chopper. Although they have been used with X rays, crystal interferometers work equally well with neutrons⁴. The interferometer is used as a phase sensitive neutron switch, in which a crystal translation of 0.96 Å is sufficient to redirect the beam. At larger translational amplitudes the interferometer has a corresponding multiplex advantage over other neutron choppers. Two of these interferometric neutron choppers could be used to construct a Fourier transform neutron spectrometer with 1,000

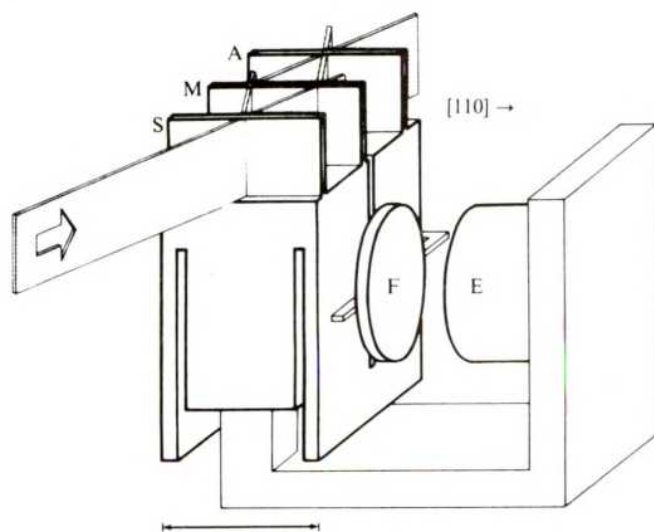


Fig. 1 The monolithic scanning X-ray interferometer which is cut from a perfect single crystal of silicon. The ferrite disk F is glued to a U-bracket which drives the elastic transverse stage and forces are generated by the electromagnet E. X-ray beam paths through the interferometer are indicated by shaded areas. 220 Bragg reflections provide beam steering in the splitter crystal S, mirrors M and analyser A. The interferometer is described in detail elsewhere⁵. Scale, 10 mm.

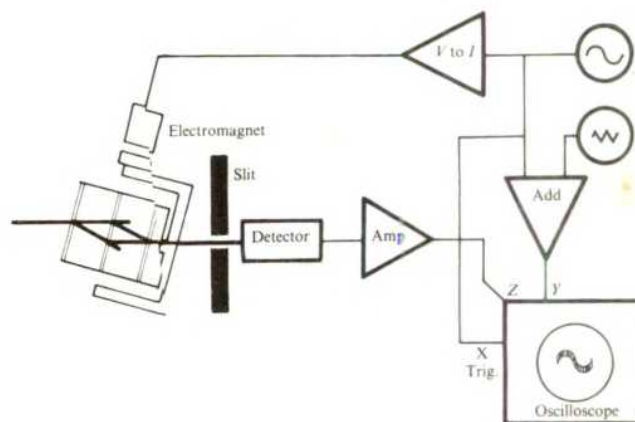


Fig. 2 The electronic drive system showing how the oscilloscope display is generated

times the resolution of competing spectrometers. With a pulsed source a single interferometric chopper could be used to construct a time of flight spectrometer which would provide high resolution even with fast neutrons.

The interferometer construction is shown in Fig. 1. The monolithic scanning interferometer^{5,6} is driven by the force between the electromagnet E and the ferrite disk F. The three wafers S, M, A which form the X-ray or neutron interferometer, are all part of the same monolithic perfect crystal. Coherent beam steering is achieved with the 220 and $\bar{2}\bar{2}0$ Bragg reflections indicated in the ray diagram. A is connected to S and M through the monolithic elastic transverse^{5,7} which allows the translational degree of freedom of A parallel to the [110] direction. When the analyser A travels a distance of 1.92 Å in the [110] direction which is normal to the Bragg planes, one fringe is observed in each of the two output beams.

In the case of zero absorption the output beams are complementary (conservation of photons or neutrons in the analyser A). For example, when the analyser translation x is pd the forward beam T has maximum intensity, whereas for $x = (p + \frac{1}{2})d$ the intensity maximum is in the deviated R-beam. Here, p is an integer and d is the Bragg spacing: 1.92 Å for the 220 Bragg reflection from silicon. The intensity N is given by

$$N = N_0 + N_1 \sin(2\pi x/d)$$

If $\dot{x} = 2 \text{ mm s}^{-1}$, which is easily achieved, then we would observe complementary output beams, amplitude modulated at 10 MHz, and with 100% duty cycle as no neutrons are absorbed in the interferometer. In practice, the fringe contrast observed with X rays may be 0.95 while for neutrons^{5,8} the best contrast observed has been approximately 0.85. Both would be 1.00 in an ideally perfect interferometer with zero absorption.

In considering the design and use of mechanical systems, one needs to know the system response to disturbances. The intensities available from both X-ray and neutron sources are so low that we could not measure the impulse response directly. We were, however, able to measure the simple harmonic frequency response directly with X rays using 30-s integration time.

Figure 2 shows the experimental arrangement and Fig. 3 demonstrates the fringes observed near a mechanical resonance in the system at 10.8 kHz. The fringe resolution is limited to about 500 ns by the time base trigger-jitter and by the frequency response of the oscilloscope z-modulation. When operating near the fundamental translational resonance which occurs at 1,420 Hz we found no loss of fringe contrast, which indicates that only the pure translation mode was excited.

The interferometer was tested with molybdenum $K\alpha_1$ radiation for which the available intensity from a conventional laboratory source is a few thousand photons per second. Each photon detected (Fig. 2) produces a bright spot on the oscilloscope screen. With 3,000 speed polaroid film it was necessary to pulse the z-modulation for 500 ns to record an image and this

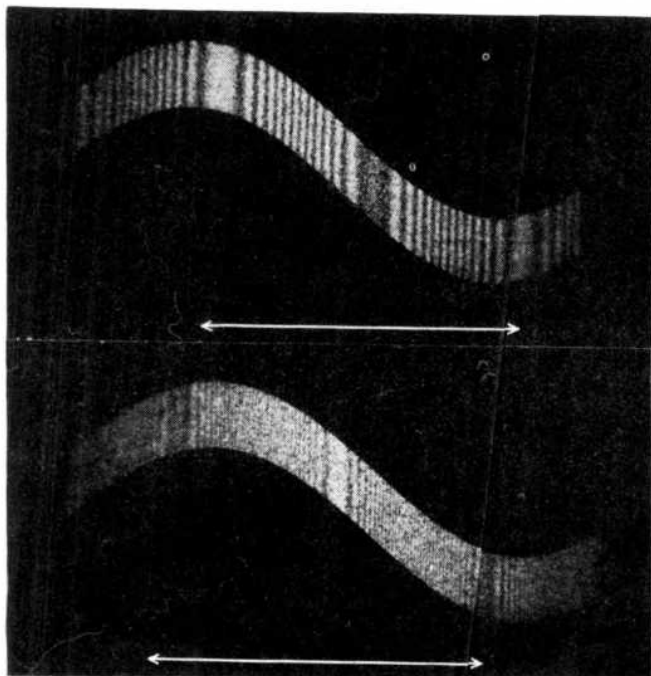


Fig. 3 X-ray fringes produced by the system outlined in Fig. 2. The finest fringes correspond to 500 ns chopping times and resolution is mainly limited by the oscilloscope performance. Each bright spot in the photograph corresponds to one detected X-ray photon. Scale, 100 μ s.

limits the present time resolution of the detector system. In Fig. 3, the interferometer was driven with a sinusoidal current of frequency ω . In a practical neutron chopper pulsed or triangular waveforms might also be used. So that the fringe system is easily visualised, the y -displacement consists of the sum of the electromagnet drive voltage and an incommensurate triangular voltage. The observed fringe spacing is, of course, not constant. The applied force is proportional to the square of the magnet current, and the observed fringes show the displacement as a function of time. The force F is given by

$$F = KI_0^2 \sin^2 \omega t = \frac{1}{2} KI_0^2 (1 + \sin 2\omega t)$$

where K is a constant and the force repetition rate is seen to be twice that of the driving current. This is clearly seen in Fig. 3 where the periodicity of the fringe pattern is twice that of the current signal. Since one fringe occurs for a displacement of 1.92 $\text{\AA}$ we were able to trace the complete frequency response of the chopper from these photographs. Thus, amplitude, stored energy and phase shift can all be measured as functions of frequency. The two fringe patterns were obtained near the 10.8 kHz mechanical resonance with only 15 mW of magnet power. The phase shift between the mechanical motion and the electromagnetic force near resonance can be clearly seen as can the ~ 500 ns spacing of the finest visible fringes. At that point the speed of the analyser wafer is 1.92 $\text{\AA}$ per 500 ns or 0.4 mm s^{-1} .

It is not unreasonable to use up 1,000 times more power in the driving electromagnet. With only 15 mW of power, the present chopper is already faster than any alternative. There are two ways in which the potential performance can be exploited. Either high power interferometric neutron chopping can be achieved on the sub-ns timescale or very precise neutron gating could be attempted on the μ s and longer timescales. The former would be attractive for extreme resolution multiplexed neutron spectroscopy using high flux neutron sources; the latter might be more attractive at pulsed sources, such as the spallation neutron source.

In future developments it will be desirable to have both positive and negative force generators. Experiments using double magnet systems and permanent magnets in place of the ferrite disk are in progress. Both systems will permit push-pull operation. The strain gradients inherent in systems which rely on

Bragg reflections from sets of standing or travelling ultrasonic waves³ have caused great problems. Because of the neutron samples' spatial and/or temporal crystal inhomogeneities, these systems are limited to frequencies well below 100 kHz with crystal thicknesses of the order of 1 mm. Provided that the interferometer wafer S, M and A undergo pure uniform translation there is no similar limit on their performance as each crystal component remains homogeneous and strain-free. The neutrons are, of course, Doppler shifted in the analyser and this is equivalent to rotating the analyser. At 2 mm s^{-1} the rotation for thermal neutrons is ~ 0.5 arc s but the Bragg angle 2θ is not changed to first order. Since the range of Bragg reflection is ~ 2 arc s at 2 $\text{\AA}$ wavelength, the chopper could be 'returned' by rotating the analyser. At much higher crystal speeds it may become necessary to correct for the second order Doppler shift in the Bragg angle by changing the temperature of the analyser crystal. Higher order Bragg reflections can be used to provide multiplexed channelled spectra and to achieve even faster chopping rates.

At present the best on-off ratio for neutrons^{4,8} is at least 6 but with more perfect crystals this should exceed 10. Although the new chopper might have advantages in experiments to determine h/M for the neutron⁹, and in providing precisely sinusoidal neutron modulation in Fourier time-of-flight spectrometers¹⁰; other possible applications are still only in the discussion stage.

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Hydrogen bond dynamics in solution

THE key to understanding the broad but featureless $\nu_s(XH)$ absorption bands of hydrogen-bonded species in solution has been the recognition by Bratos that the $\nu_s(XH \cdots Y)$ hydrogen bond stretching mode has a pronounced stochastic character, and that the $\nu_s(XH)$ mode rapidly loses its phase coherence as a result of its strong anharmonic coupling to the $\nu_s(XH \cdots Y)$ mode¹. This idea has been further developed by Yarwood and Robertson^{2,3}, who treat the dynamics of the ν_s mode more precisely than does Bratos. The most detailed prediction of these theories is that the amplitude of modulation of the $\nu_s(XH)$ mode should be proportional to the r.m.s. amplitude of the ν_s displacement coordinate r_2 , and should thus vary with temperature as $(\coth(\hbar\omega_2/2kT))^{1/2}$, where $\omega_2 (= 2\pi c\bar{\nu}_s)$ is the angular frequency of the ν_s mode. We report here an experimental confirmation of this prediction for a dilute solution of self-associated CD_3OH in freon 11. We also investigate how the dynamics of the hydrogen bond vibration change as the solvent freezes, and we present evidence that at liquid helium temperature the $\nu_s(XH \cdots Y)$ mode can be regarded as a quantum-mechanical stochastic oscillator executing a strongly overdamped zero-point motion.

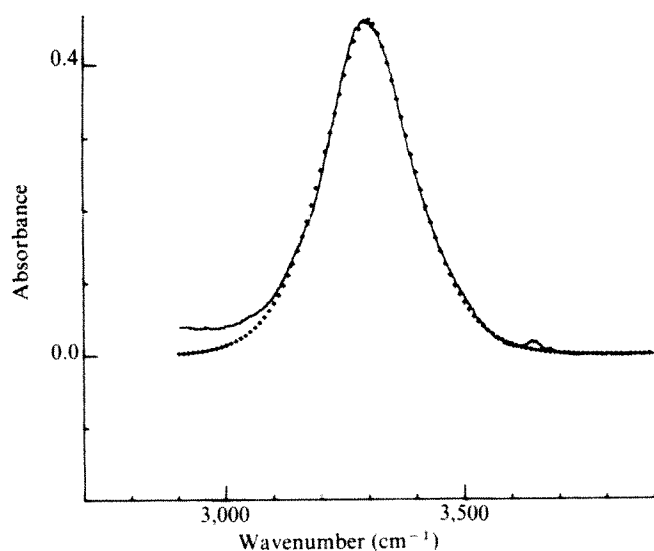


Fig. 1 Observed (continuous line) and fitted (crosses) spectrum of self-associated CD_3OH in CFCl_3 ; temperature, 240 K; concentration, 0.2 M. There is a further broad absorption band on the low frequency side, not accounted for by the theory, which may be due to multimeric species. This makes it difficult to determine the low frequency baseline satisfactorily. The absorption band at $3,650\text{ cm}^{-1}$ is due to free CD_3OH ; its intensity increases with increasing temperature. The data were zero-weighted below $3,100\text{ cm}^{-1}$ and between $3,625$ and $3,675\text{ cm}^{-1}$ during curve-fitting.

Yarwood and Robertson^{2,3} assume that the ν_o displacement coordinate r_2 satisfies the Ornstein–Uhlenbeck stochastic differential equation⁴

$$\ddot{r}_2 + \gamma \dot{r}_2 + \omega_2^2 r_2 = m_2^{-1} F(t) \quad (1)$$

and, by applying Kubo's theory of a randomly modulated oscillator⁵, show that the autocorrelation function of the $\nu_s(\text{XH})$ transition dipole moment is

$$\phi(t) = \exp \left\{ -\Delta^2 \int_0^t (t-x) \psi(x) dx \right\} \quad (2)$$

where ψ is the autocorrelation function of the Ornstein–Uhlenbeck stochastic process⁴. The amplitude of modulation Δ of the ν_s mode is given by

$$\Delta = K_{112} \langle r_2^2 \rangle^{1/2} / m_1 \omega_1 \quad (3)$$

where K_{112} is an anharmonic coupling constant, and the characteristic time τ_c of the modulation is given by

$$\tau_c = \gamma / \omega_2^2 \quad (4)$$

Equations (2)–(4) give a good account of the experimental $\nu_s(\text{XH})$ absorption band profiles of phenol–acetonitrile and phenol–dioxan complexes in solution in carbon tetrachloride, correctly predict the changes which occur on deuteration, and enable the effect on the bandshape of changing the solvent to be understood⁶.

The most stringent way to test the theory, however, is to study the temperature dependence of the $\nu_s(\text{XH})$ bandshape⁷. According to equations (3) and (4), Δ should vary as $(\coth(\hbar\omega_2/2kT))^{1/2}$, while τ_c , being proportional to the microscopic viscous damping constant γ , should increase rapidly as the temperature is reduced. To investigate this, we have recorded digitally the $\nu_s(\text{XH})$ absorption bands of self-associated CD_3OH in freon 11 (CFCl_3) between 280 and 160 K, and have estimated the parameters Δ and τ_c by a nonlinear least-squares analysis of the bandshape. A typical $\nu_s(\text{XH})$ band with its theoretical reconstruction superimposed is shown in Fig. 1, and a summary of the parameter estimates is given in Table 1. The method of analysis was similar to that described previously⁶,

except that $\bar{\nu}_o (= \omega_2/2\pi c)$ was treated as a fixed parameter with a value of 100 cm^{-1} . This is a compromise between the value of 80 cm^{-1} (or at most 100 cm^{-1}) suggested by Lake and Thompson⁸, and that of 120 cm^{-1} found by Zelsmann (personal communication).

The regression $\Delta = a + b (\coth(\hbar\omega_2/2kT))^{1/2}$ is close to linear, with a correlation coefficient of 0.99. The intercept a does not differ significantly from zero at the 95% confidence level. This strongly supports equation (3), and shows that the contribution to the broadening from mechanisms other than vibrational phase relaxation is small. As expected, the viscous damping constant γ increases with decreasing temperature; however, the temperature dependence of γ cannot be well represented by a law of the form $\exp(A/kT)$, particularly at the lower temperatures. (Although this may be partly due to the uncertainty in the value of ω_2 , previous work⁶ has shown that γ is not related to the macroscopic viscosity of the solvent.) The Kubo parameter $\tau_c\Delta$ increases somewhat as the temperature is reduced, but the system is far from the slow modulation limit ($\tau_c\Delta \gg 1$) at all temperatures above 160 K, and the $\nu_s(\text{XH})$ absorption band profiles differ markedly from gaussian shape.

Below 160 K the solution freezes to form a vitreous solid. On freezing, the bandshape changes dramatically: the halfwidth at 170 K is 175 cm^{-1} , and at 150 K it is 210 cm^{-1} (see Fig. 2). However, analysis of the bandshape shows that the amplitude of modulation continues to decrease, in a manner consistent with equation (3), as the temperature is reduced to 150 K: there is no discontinuous change in the amplitude of modulation at the transition point, even though the $\bar{\nu}_s(\text{XH})$ frequency abruptly shifts upwards by several wavenumbers on freezing. (The general trend is for the $\nu_s(\text{XH})$ frequency to shift steadily downwards with decreasing temperature.) On the other hand, τ_c increases from 0.046 ps to 0.40 ps, $\tau_c\Delta$ from 0.83 to 7.0, and $\gamma/2\omega_2$ from 0.44 to 3.9.

Although these estimates are imprecise, particularly at the lower temperature where the errors are of the order of 10%, they do show qualitatively how the dynamics of the hydrogen bond change as the solvent freezes. In the solid the $\nu_o(\text{XH} \cdots \text{Y})$ vibration is heavily overdamped, and relaxes slowly. The large value of the Kubo parameter $\tau_c\Delta$ indicates that the random frequency modulation is quasi-static (that is, that $\phi(t)$ relaxes in a time τ_r much shorter than the characteristic time τ_c of the modulation), so that the $\nu_s(\text{XH})$ absorption band is nearly gaussian in shape. The spectacular increase in halfwidth on freezing can be entirely attributed to a decrease in the kurtosis of the spectrum on entering the slow modulation regime.

On further cooling to 6 K the $\nu_s(\text{XH})$ band remains gaussian in profile and the halfwidth decreases to 190 cm^{-1} ; the amplitude of modulation at 6 K is estimated by analysis of the spectrum to be 81 cm^{-1} . Although this is significantly greater

Table 1 Bandshape parameters for self-associated CD_3OH in CFCl_3 (0.2 M solution)

T/K	$(2\pi c)^{-1} \Delta\text{ cm}^{-1}$	$\tau_c\Delta$	$\gamma/2\omega_2$	$[\coth(\hbar\omega_2/2kT)]^{1/2}$
280	120.9	0.60	0.25	1.99
270	118.3	0.63	0.26	1.96
260	115.9	0.64	0.28	1.92
250	114.0	0.68	0.30	1.89
240	111.7	0.70	0.31	1.85
230	110.1	0.72	0.33	1.82
220	105.7	0.78	0.37	1.78
210	101.8	0.77	0.38	1.74
200	99.9	0.79	0.40	1.70
190	98.6	0.80	0.41	1.66
180	96.8	0.82	0.43	1.62
170	95.4	0.83	0.44	1.58
160	94.2	0.84	0.45	1.54
150	92.9	7.0	3.9	1.50
6	80.6	8.0	5.0	1.00

The figures in the last two columns assume that α_2 is known, and are subject to possible systematic error.

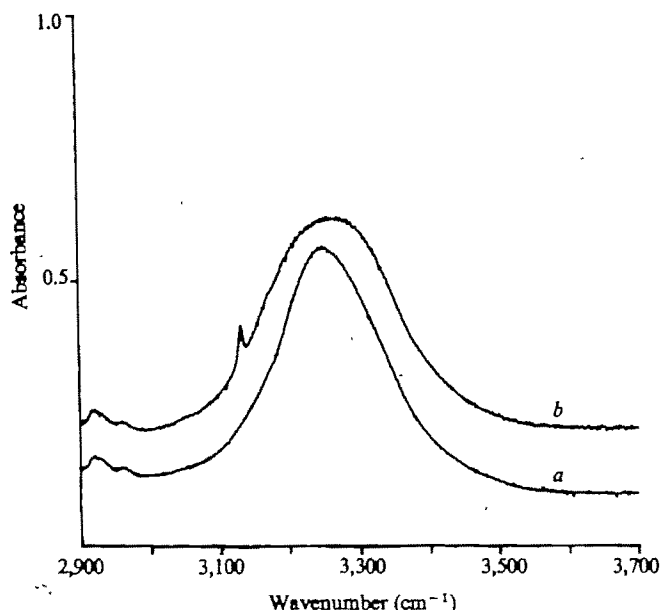


Fig. 2 A comparison of the $\nu_s(\text{XH})$ absorption spectra at 170 K (liquid) (a) and 150 K (solid) (b). The halfwidths are 175 cm^{-1} and 210 cm^{-1} , respectively, but the amplitudes of modulation are 95 and 93 cm^{-1} . The spectrum of the solid solution is very nearly gaussian in profile. The feature at $3,130\text{ cm}^{-1}$ is an uncompensated solvent band, which is narrower and more prominent in the solid. The curves have been offset for display.

than the 95% confidence estimate $58 \pm 6\text{ cm}^{-1}$ obtained by extrapolating to zero temperature the relationship

$$\Delta = a + b[\coth(\hbar\omega_2/2kT)]^{1/2} \quad (5)$$

with parameters $a = (-4 \pm 10)\text{ cm}^{-1}$ and $b = (62 \pm 6)\text{ cm}^{-1}$ determined from measurements on the liquid between 160 K and 280 K, at least part of the discrepancy may be due to error in ω_2 . At 6 K the random frequency modulation of the $\nu_s(\text{XH})$ band is largely produced by the zero-point motion of the $\nu_s(\text{XH}\cdots\text{Y})$ mode.

Very similar results, consistent with equations (3) and (5), have been obtained by analysing the spectrum of a 0.2 M solution of CH_3OD in freon 11 over the same temperature range. The ratio $b(\text{H})/b(\text{D})$ (see equation (5)) is found to be approximately $\sqrt{2}$, in agreement with theory. However, the transmission window (or 'Evans hole') at about $2,460\text{ cm}^{-1}$ in the spectrum of self-associated CH_3OD complicates the analysis and leads to various uncertainties.

We believe that we have observed an unusual phenomenon: the zero-point motion of a heavily overdamped stochastic oscillator. Because of the strong damping and consequent slow relaxation of the r_2 coordinate in the frozen medium, the $\nu_s(\text{XH})$ transition dipole moment relaxes in a time which is small compared with τ_0 . The $\nu_s(\text{XH})$ band thus reflects only the probability distribution of the r_2 displacement coordinate, which is gaussian. The halfwidth of the band at liquid helium temperature is consistent with the variance of this displacement coordinate being $\hbar/2m_2\omega_2$. It must be admitted, of course, that the theory in its present form^{2,3} is semi-classical, and not strictly valid at liquid helium temperatures. The theory could be made rigorous by simply replacing the classical Ornstein-Uhlenbeck autocorrelation function $\psi(t)$ in equation (2) by an autocorrelation function derived from a quantum-mechanical model of a brownian oscillator⁹. Because in the disordered solid phase the conditions for slow modulation are well satisfied, so that the bandshape depends only on the probability distribution and not on the dynamics of the modulation process, there seems little to be gained by such a refinement.

The broad $\nu_s(\text{XH})$ bands of solutions of hydrogen-bonded species at low temperatures have sometimes been interpreted in terms of frozen-in disorder. Our work suggests that in dilute

solid solution this disorder should not be understood as a distribution of $\text{XH}\cdots\text{Y}$ equilibrium distances, but rather in terms of the slow relaxation of the r_2 coordinate back to its equilibrium position and the absence of any oscillatory character to its motion (see refs 1, 7). If there were a distribution of $\text{XH}\cdots\text{Y}$ bondlengths in dilute solid solution, it is improbable that this distribution would be gaussian or that it would produce a broadening of the $\nu_s(\text{XH})$ band at liquid helium temperature consistent with equation (3). Perhaps the most convincing evidence in favour of our interpretation, however, is the absence of any discontinuity in the amplitude of modulation at the freezing point, despite the pronounced change in the shape of the absorption band.

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Origin of olivine subgrain boundaries in mantle peridotites

INVESTIGATION of the fabric, texture and dislocation microstructure of olivine has given much insight into the deformation history of mantle peridotites¹. Optically visible (100) subgrain boundaries (tilt or kink-band boundaries) are particularly significant in this respect. Their existence is the principal argument favouring intracrystalline glide as the mechanism of deformation² and the spacing between them (d_{100}) is considered to be a measure of the stress conditions during deformation³⁻⁶. Previous workers have argued that the sub-boundaries in olivine originated at the onset of deformation of peridotites^{1,2}, pre-dating both the recrystallised strain-free secondary grains⁷ and grain boundary development in general¹. However, re-examination of the distribution of sub-boundaries in olivine reveals features which are quite inconsistent with this interpretation. It is suggested in the present work that (100) sub-boundaries are a late feature in these peridotites and most probably have no connection with hypothetical asthenosphere flow.

The slip system activated during the development of (100) subgrains in olivine is $\{0kl\} [100]$ (ref. 8); the slip plane is usually subparallel to (010) and the slip direction is always $[100]$ (Fig. 1). Detailed thin section examination of mantle peridotites shows that the point of inside termination of a (100) sub-boundary (the point nearest to the common rotation axis of the two related subgrains; I intersection in Fig. 1) commonly coincides with a local convex feature on the grain boundary (Figs 2 and 3). The latter may be the corner of a polygonal or cusp-like re-entrant of the deformed grain or the point of maximum curvature of a smooth curvilinear boundary.

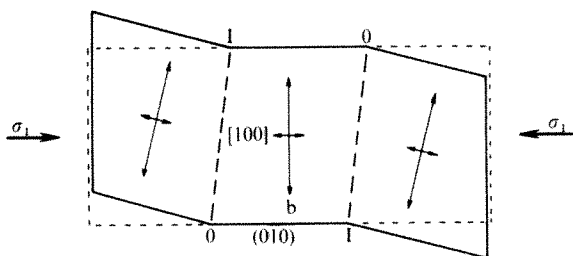


Fig. 1 Development of (100) subgrain boundaries in olivine by intracrystalline glide. (010), Glide plane; [100], glide direction; broken lines, original rectangular grain outline; σ_1 principal stress axis; I, subgrain and grain boundary intersection nearest to common rotation axis of two related subgrains.

The intersection of subgrain and grain boundaries at triple points, with reported 120° dihedral angles has been noted previously², and used as evidence for subgrain boundary development after (100) sub-boundary formation. Textural evidence contradicting this position is as follows. (1) Most olivine grains in peridotites have curvilinear to sinuous grain boundaries and re-entrants are just as cusped on grain boundaries parallel to sub-boundaries as on grain boundaries normal to sub-boundaries. 'Dihedral angles' are not in any way exclusively or even predominantly associated with the intersection of subgrain and grain boundaries. (2) The actual dihedral angles vary markedly in magnitude and show no indication of having adjusted, through recrystallisation, to equilibrium values. (3) Cusp-like re-entrants at triple points are predominantly associated with I intersections (Fig. 1) whereas re-equilibration of grain boundary energy through recrystallisation must result in a nearly equitable distribution between triple points associated with both I and O intersections (Fig. 1). (4) (100) Sub-boundaries frequently have uneven spatial distribution within individual grains, which, in itself, strongly suggests that they are localised by the convex features on the grain boundaries. (5) Sub-boundaries in primary (large) olivine grains also intersect convex features on secondary grain boundaries, and, although they are discontinuous in largely or wholly enclosed secondary grains, they do not have continuity across them. Hence, secondary olivine grains also seem to predate sub-boundary development.

Further evidence for the late development of sub-boundaries is suggested by their abundance in the elongated olivine grains of foliated porphyroclastic and equigranular peridotites⁷. The stress system required for (100) subgrain formation (Fig. 1) and the resulting deformation, shortening parallel to [100], are entirely inconsistent with the proposed deformation mode of these peridotites, translation gliding on $\{0kl\}$ [100] (refs 1, 2, 9). An attempted explanation of this inconsistency in the literature² is quite unconvincing, since the net elongation of the olivine grain under consideration is due to grain rotation, not subgrain formation. Moreover, olivine grains in these peridotites would continue to elongate with continued deformation, so that early formed sub-boundaries should become smeared and irregular. In fact, as is well known, (100) sub-boundaries are usually sharply defined and linear.

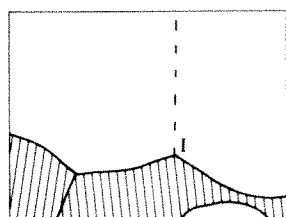
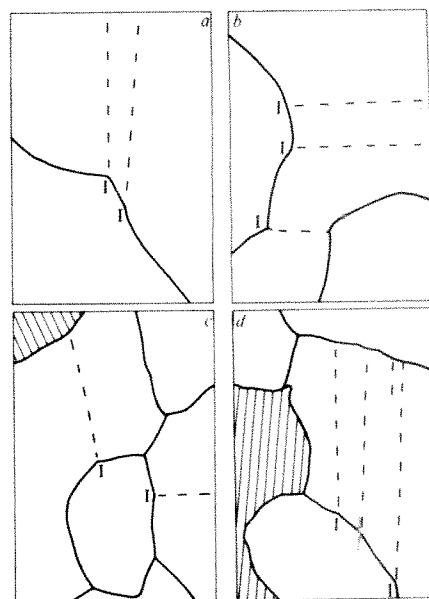


Fig. 2 (100) Sub-boundary in peridotite (spinel lherzolite) nodule from Camperdown, Victoria, Australia. Unshaded grains, olivine; shaded grains, orthopyroxene; I, I intersection (Fig. 1).

I suggest that the sub-boundaries in olivine grains of mantle peridotites developed during a later phase of deformation than that which produced the overall rock fabric. As presently envisaged, this later deformation was most probably associated with structural disruption leading to inclusion of the peridotite xenoliths in the basalt liquids. The sub-boundaries are nucleated at the convex features of appropriately orientated grain boundaries, their development being activated by the local stress concentration expected at these points. The role of stress concentration at the tip of Griffiths cracks in the brittle fracture of solids is well understood¹⁰. A direct analogy with the development of (100) sub-boundaries is the nucleation of annealing twins in face centred cubic metals¹¹. The distribution of sub-boundaries in a peridotite does not reflect the overall stress on the rock, but the local stress on individual grains. Hence, the preferred orientation of sub-boundaries in foliated peridotites is inherited from the preferred orientation of the olivine grains themselves. In undeformed peridotites the sub-boundaries are randomly orientated. The lack (or minimal development) of (100) sub-boundaries in secondary olivine grains is attributed to their smaller size and more regular and equidimensional shape, which allows them to resist deformation by subgrain formation much more readily than the larger, more irregular and highly crenulated primary grains. Similarly, partly or wholly included secondary grains appear passive to the deformation not because they postdate it but because they are inappropriately orientated to allow translation gliding on the same system as the host.

Fig. 3 (100) Sub-boundaries in Camperdown spinel lherzolite (see Fig. 2).



Recent d_{100} calculations of the stresses under which mantle peridotites have been deformed give values about one order of magnitude greater than that derived from plate tectonic models on the rheology of the mantle^{5,6}. This discrepancy has led to the suggestion that the deformation causing subgrain development occurred during diapiric upwelling of the peridotite rather than during horizontal asthenospheric flow⁵. This is essentially consistent with the present report, although the possible deformation mechanism is not necessarily limited to diapiric upwelling. Grain boundary stresses developed immediately before magma incorporation might be sufficient for subgrain development. Should this be the case, the presence of (100) sub-boundaries would not be a useful criterion to distinguish between exotic and cognate xenoliths¹². Quite clearly, the crystallisation and deformation histories of peridotites preserved as

nodules in basalts are far more complex than had been anticipated in the not-too-distant past, and the extent of preservation of asthenospheric flow characteristics in them, if indeed such ever existed, remains to be demonstrated.

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Some new measurements on Stonehenge

THE possible astronomical significance of Stonehenge has made it desirable to make new measurements of some of its features, and I report here the results of a survey made in April 1978. New values are given for the axis of symmetry of the Avenue, the figure of the four Stations, the original position of the peak of the Heel Stone and the axis of symmetry of the sarsen trilithons (Fig. 1).

Linear measurements were taken to the nearest 5 mm with a Fibron glass-fibre tape at constant tension, calibrated against a steel tape kept as a standard. Angular measurements were made with a theodolite reading directly to 20" and by estimation to 5". The coordinate system used (Tables 1-3) has an origin 141.42 m south-west of the bronze peg β close to the centre of Stonehenge¹, with axes directed to true north and east, so that the coordinates of this peg are 100.00 E, 100.00 N. This system of local coordinates is to be preferred to National Grid coordinates, because it allows direct conversion to true azimuths. Heights are given in metres above Ordnance Datum, the level of the bench mark on stone 16 being taken as 103.11 above OD. The full National Grid coordinates of the peg β are SU 12247.43, 42195.20 (ref. 1). The referring object at the peg β was the west side of a prominent square brick chimney at Larkhill (National Grid reference 12750, 44530), the azimuth of which had been determined by Thom in 1973 as 12°10'24" (personal communication).

The part of the Avenue surveyed was the straight section which runs downhill northeastwards from the entrance of the Stonehenge earthwork. Its length to the point where it begins to change direction eastwards is 509 m. Eighteen transverse profiles were measured with a staff and level to find the lowest point of the surface over the ditch on either side, and a peg was inserted halfway between each of these pairs of points. The first of these profiles was about 2 m from the beginning of the Avenue and the last about 16 m from the end of the straight section. The remainder were spaced about 30 m apart, except where the modern road (A 344) crosses the Avenue. The coordinates of the index-marks on the pegs were then determined by a theodolite traverse. The closing error over a distance of 982 m was 0.087 m, giving a consistency of measurement of better than 1 part in 10,000. The adjusted coordinates of the 18 pegs A-T are shown in Table 1. The traverse began and ended at peg A.

Regardless of the method adopted for estimating the azimuth of the straight line which is the 'best fit' to these 18 points, the

result is extremely close to 49°54'40", and this is accordingly taken to be the azimuth of the axis of symmetry of the straight section of the Avenue. This value has been confirmed by direct observations of the azimuths of the lines joining the ends of the two Avenue ditches. At the Stonehenge end these were excavated by Hawley in 1923 (ref. 2), and were located by probing for the lowest point of the ditch bottoms in 1978. At the far end, where the Avenue begins to change direction, both ditches were exposed by excavation in 1978. The azimuth of the north-west ditch, end to end, is 49°52'19", and that of the south-east ditch 49°56'5", both values being the mean of six observations. The mean of these two values, 49°54'12", agrees well with the calculated axis.

This new value differs significantly from the mean axis of 49°35'51" found by Lockyer³, and from the axis of 49°34'18" which he adopted for reasons which can now be seen to be irrelevant. Lockyer used only six pegs on the apparent centre line of the Avenue, and did not record their positions in detail. Two of them were 140 ft apart "near the commencement of the avenue", and four others "at distances averaging 100 feet apart nearer the further recognisable extremity". Elsewhere, he states that the Avenue was discernible "for more than 1,300 ft from the centre of the temple". This implies that his furthest peg lay not more than 425 m from the centre of Stonehenge. The straight section of the Avenue continues, however, for a further 139 m beyond this point, although it has been somewhat reduced by cultivation.

It is no longer necessary to attempt to derive the date of construction of this section of the Avenue from its orientation, as Lockyer did, because there are now two closely agreeing radiocarbon dates from deer antler picks found on the bottoms of the two ditches close to Stonehenge. These are BM-1164, 1728±68 b.c. and HAR-2013, 1770±70 b.c. The weighted mean of these dates, when corrected⁴, is 1265±80 b.c.. At this date the first gleam of the summer solstice sunrise (with 2' of the upper limb above the horizon) would have had an azimuth of 49°30', which differs from the axis now determined by about 25'

Fig. 1 Diagrammatic plan of Stonehenge, showing the beginning of the Avenue, the Heel Stone, the four Stations (91-94), the sarsen circle and horseshoe, and the survey point β . Standing stones are shown in black, fallen and missing stones in outline.

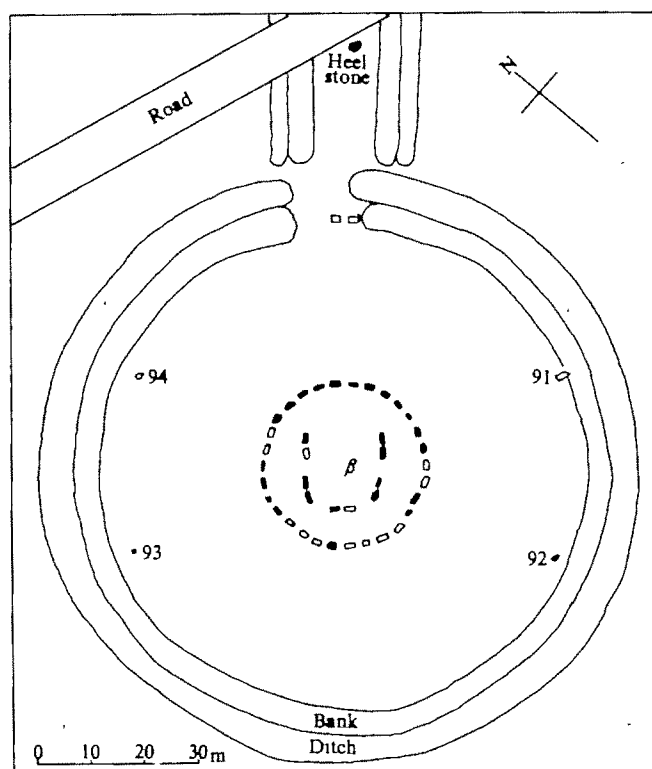


Table 1 Coordinates of Stonehenge centres and midpoints of the Avenue

Survey point	Eastings	Northings	Height
Centre of Aubrey Holes	99.58	99.37	
Centre of sarsen circle	99.15	99.61	
β	100.00	100.00	102.33
A	143.37	137.55	101.25
B	155.40	147.62	100.70
C	178.56	166.52	99.95
D	197.87	183.59	99.12
E	220.32	203.07	97.79
F	243.33	222.05	96.53
G	266.14	241.38	95.24
H	288.97	260.56	94.07
J	311.81	279.78	93.26
K	334.76	298.75	92.61
L	357.53	317.98	92.05
M	380.27	337.24	91.53
N	403.36	356.10	91.41
P	425.63	375.87	91.50
Q	448.61	394.88	91.31
R	471.65	413.80	90.96
S	494.41	433.04	90.53
T	519.00	453.54	89.60

If, however, there were trees growing on the skyline to a height of 10 m, which is by no means impossible, the azimuth of the apparent sunrise thus defined would have almost precisely matched the azimuth of the axis now determined.

If it is assumed that the lines of the Avenue ditches were laid out by lateral offsets from a central axis, and that this axis passed through the point defined by the means of the coordinates of the points A–T, then the axis prolonged southwards would have passed 1.46 m north of the peg β , 1.14 m north of the centre of the sarsen circle and 1.74 m north of the centre of the Aubrey Holes¹. The centre of the unfinished double circle of bluestones of period II (ref. 5) is not known precisely, because only a small proportion of its stoneholes have so far been excavated; however, the available evidence suggests that it was close to the centre of the subsequent sarsen circle. These divergencies imply, therefore, that the double bluestone circle and the Avenue, both of period II, did not share a common axis of symmetry.

The four Stations lie approximately on the circumference of the circle of Aubrey Holes, and are often stated to have formed a rectangle with sides aligned on extreme azimuths of the rising of the Sun and Moon⁶. Until the present survey, however, the position of Station 94 was located only by inference or conjecture. The values given here (Tables 2 and 3) are derived from direct observation and measurement on the ground.

Station 91 is represented by a fallen stone, now leaning outwards against the inner slope of the bank, with an overall length of 2.75 m. The original position of its centre is best estimated from the outline of the steep-sided stonehole in which it stood. This was found by probing in April 1978.

At Station 92, the centre of the deepest part of the stonehole can be determined from an unpublished plan made after its excavation in 1921 (ref. 7), and was confirmed by probing in July 1973 and again in April 1978.

Station 93 is occupied by the stump of an upright stone, but the dressing of parts of its sides below present ground level (and well below prehistoric ground level), like that of the sarsen stones of period IIIa, suggests that it may be a later replacement of an earlier marker. The other surviving Station stone, no. 91, shows no signs of dressing on its visible surface. The centre of stone 93 was determined by direct measurement.

The stonehole at Station 94 was located by excavation for the first time in April 1978, when the gravel access-path beneath which it lies was being replaced by turf. The filling was removed only far enough to define the upper edge of the hole, at the surface of the chalk, but probing showed that the sides descended almost vertically. Its eastern margin, where it may overlap

the adjacent Aubrey Hole 46, was left undisturbed. This Aubrey Hole was probably excavated, in part at least, by William Cunnington on behalf of Sir Richard Colt Hoare early in the nineteenth century. The latter records the finding of a cremated human burial⁸, many of which have been found in the other excavated Aubrey Holes⁹.

The coordinates of the apparent centres of the four Stations, the included angles at each, and the lengths and azimuths of the sides of the quadrilateral thus defined are given in Tables 2 and 3. These values suggest that the figure of the four Stations could have been intended to be a rectangle, with adjacent sides and diagonals roughly in the ratio of 5:12:13, the Pythagorean triangle suggested by Dibble¹⁰. From the evidence available, however, it is impossible to determine the precise shape, size or orientation of the original figure, because it cannot be assumed that the peaks of the stones (the points of reference for astronomical observations) were identical in plan with the centroids of the holes in which they stood. For this reason the original azimuths may have differed from those given here by at least $\pm 1^\circ$ for the shorter sides of the figure, and by at least $\pm 30'$ for the longer. These uncertainties must be taken into account in any calculation which is made with the aim of showing that the four Stations had an astronomical significance.

The Heel Stone now leans very markedly from the vertical, although this has sometimes been ignored in discussing its astronomical significance¹¹. By a combination of direct measurement and photogrammetry, its inclination has been determined as $24^\circ 26'$ from the vertical, in a plane bearing $233^\circ 22'$ from true north. The coordinates of its peak, as seen today from the centre of Stonehenge (peg β) are 160.21° E, 148.75° N, 105.07 m above OD. If it were set vertical, its present apparent peak would lie a little beyond and below its new apparent peak, the coordinates of which would be 161.94° E, 150.04° N, 105.60 m above OD.

The Heel Stone is part of the structure of period I, for which the best estimate of the centre is that of the circle of Aubrey Holes (Table 1). The ground levels within the stones of Stonehenge have been altered during the last 25 yr by re-turfing, by the laying of gravel in 1963 and by its replacement with turf in 1978; but extrapolation from adjacent contours on undisturbed ground suggests that in the absence of disturbance the ground level at the Aubrey centre would today be 102.38 m above OD. If an allowance of 0.56 m is made for the weathering of the chalk since the building of Stonehenge I (ref. 12), and of 1.55 m for the eye-height of a prehistoric observer¹³, the level of a prehistoric observer's eye would have been at 104.49 m above OD. From this position the azimuth of the peak of the upright Heel Stone would have been $50^\circ 54'$, and its altitude would have been $47^\circ 29'$, so that 0.3 m of its top would have projected above the bare horizon. Once more, however, if there were trees on the skyline standing more than 10 m high, the peak of the stone would not have projected above the apparent horizon for an

Table 2 Coordinates and included angles at the four Stations

Station	Eastings	Northings	Included angle
91	139.00	80.00	$89^\circ 24'.6$
92	113.03	57.80	$89^\circ 32'.5$
93	61.58	118.97	$91^\circ 2'.4$
94	86.22	140.47	$90^\circ 0'.5$
Intersection of diagonals	99.37	99.99	$45^\circ 11'.4$

Table 3 Distances and azimuths between the stations

Stations	Distance	Azimuth
91 to 92	34.17	$229^\circ 28'.5$
92 to 93	79.93	$319^\circ 56'.0$
93 to 94	32.70	$48^\circ 53'.6$
94 to 91	80.26	$138^\circ 53'.1$

observer of the summer solstice sunrise. A question posed by Hoyle thus remains unanswered¹⁴.

Thom has shown¹ that the inner faces of the outer four trilithons at ground level are a good fit to an ellipse, the major axis of which has an azimuth of $49^{\circ}57' \pm 3'$, corresponding to astronomical sunrise at 1600 ± 450 b.c. He recognised that the second, or south, trilithon (stones 53, 54) was leaning inwards, perhaps by 3° at ground level, but even so, his estimate of the axis of symmetry of the trilithons was based on the present ground plan of their inner faces.

The inclination of the second trilithon was estimated in the present survey to be $1^{\circ}5'$ from the vertical, which corresponds to an inward displacement at ground level of 0.10 m. If this is corrected, the major axis of the best-fit ellipse would be rotated anticlockwise through an angle of $25^{\circ}-30^{\circ}$, giving a revised azimuth of about $49^{\circ}30'$. This is close to the calculated azimuth of the first-gleam summer solstice sunrise (with $2'$ of the upper limb showing) in 2045 b.c., which is $49^{\circ}31'17''$. This date is the weighted mean, when corrected, of two radiocarbon dates for the Stonehenge II/IIIa transition, BM-46, 1720 ± 150 b.c. and I-2384, 1620 ± 110 b.c. The resulting inference is that the bull-dozers of the Avenue and of the sarsen structure defined sunrise by the first gleam of the upper limb of the Sun over the apparent horizon.

I thank the National Trust and their tenant, Mr J. Wort, for permission to survey and excavate on the Avenue, the Ancient Monuments Directorate, Department of the Environment, for permission and facilities for the excavation at Station 94, the Department of Civil Engineering, University College, Cardiff for the loan of a theodolite, and my wife for much patient help.

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Determination of critical variables in a microbial predator-prey system by catastrophe theory

THE differential or difference equations that are used customarily to describe predator-prey interactions can give many insights into the behaviour of simple ecosystems, but only rarely can they be used to predict the population densities of real organisms with any accuracy. Thus even given long-term observations of population densities it is often difficult to deduce very much about the behaviour of the organisms. We present here an analysis in which we use population measurements to determine the variables that govern the response of an amoebal predator to its prey. Our results support the suggestion of Curds and Cockburn¹ that it is the prey-predator ratio rather than the prey density to which the amoebae are sensitive. We have arrived at our conclusions by applying catastrophe theory² in a way that we believe will be useful in many other contexts.

Dent *et al.*³ grew vegetative amoebae of the cellular slime mould, *Dictyostelium discoideum*, in continuous culture with a bacterial food source, *Escherichia coli* B/r. Figure 1 illustrates

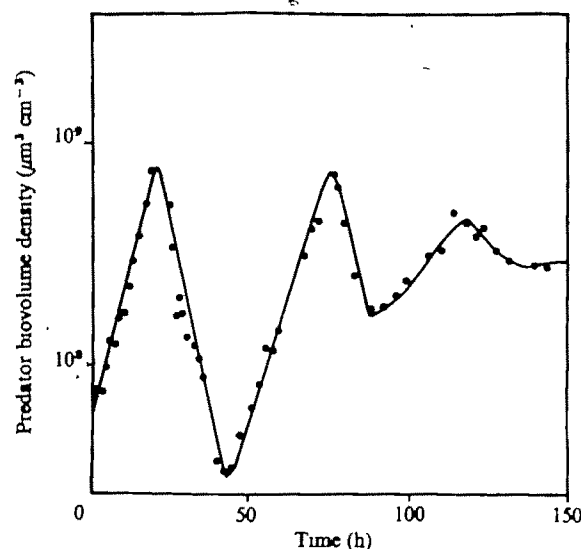


Fig. 1 Biovolume density of *D. discoideum*, data from ref. 3. The solid line is the fit obtained using the cusp catastrophe and is not significantly sensitive to the choice between the two conventions discussed in the text.

how during the first 150 h of the experiment the amoebae passed through alternating phases of exponential growth and decline. There was a very rapid change from one such phase to the next, and the specific growth rate, λ , remained constant throughout intervals of as much as a day, during which time the bacterial concentration measured in terms of biovolume (the product of the number density and the mean cell volume) could change by a factor of 100. The observation that the specific growth rate was shifting abruptly from one more or less constant value to another rather than moving smoothly in response to changes in prey density suggested that the system could profitably be studied in terms of catastrophe theory with λ as a state variable.

From Thom's list of seven elementary catastrophes we chose the cusp because it is the simplest which is consistent with the observation that in time the sudden changes in λ became smaller and eventually disappeared altogether. The cusp catastrophe requires two control variables, and we chose the culture age (a) and the bacterial biovolume density (H). With these choices a plot of prey biovolume as a function of time becomes the projection of the trajectory of the system into the control space (by system we mean the biochemical reactions which determine the behaviour of the amoebae, not the predator-prey system as a whole). By marking the points at which the abrupt changes in the state variable occurred, we were able to sketch the catastrophe set (Fig. 2). We could then relate a and H to the control variables of the canonical cusp catastrophe, and this in turn reduced the determination of λ as a function of a and H to a simple curve-fitting exercise. We then integrated λ to obtain the solid curve drawn in Fig. 1.

The curve clearly fits the data well, though we stress that the significant point is the qualitative picture, that is the nearly straight lines and the decreasing periods and slopes. The close agreement between the observed and predicted numerical values of the slopes is not evidence in favour of the model, because catastrophe theory allows us sufficient freedom to arrange this agreement providing that the qualitative behaviour is correct.

There is, however, a difficulty. Catastrophe theory explains sudden jumps in terms of shifts of the system from one stable equilibrium to another⁴. To predict where these will occur we must know not only how many stable steady states exist at a given point in the control space but also what determines which of two or more alternative steady states the system will be in. The former is deduced from the canonical form as given by Thom² and the latter is determined by specifying one of the 'conventions' of catastrophe theory. The two commonly used

conventions are the 'perfect delay' convention, in which we suppose that the system will remain in a local minimum until that minimum disappears, and the 'Maxwell' convention, in which we suppose that the system will always seek a global minimum of potential.

The catastrophe set indicated by the broken lines in Fig. 2 has the familiar cusp shape characteristic of the perfect delay convention, but the jumps occur as the trajectory enters the cusp, not as it leaves it. This implies that the system is moving away from one stable equilibrium and towards another at a higher potential. Such behaviour is not impossible to explain, but it involves a rather complicated mechanism as the system requires some separate means for choosing between steady states (P.T.S. in preparation).

We have found, however, that if we replace the control variable H by H/P , the ratio of prey to predator biovolume densities, we obtain the result shown in Fig. 3. All five jumps now lie on a single straight line, which we take as the catastrophe set of the cusp catastrophe with the Maxwell convention. This convention does not require us to postulate a separate switch, and so we deduce that the factor which determines the growth of the amoebae is more likely to be the ratio of biovolumes. This is contrary to what is assumed in most models of microbial predation but is supported by the result of Curds and Cockburn¹ who found that the feeding rate of *Tetrahymena pyriformis* was more closely correlated with H/P than with H .

We have a predator-prey system which we have not been able to model in detail and which has features inconsistent with the usual models. As a first step towards understanding the system, we want to know whether it is H or H/P to which the amoebae respond. Catastrophe theory tells us that if the critical variable is H/P , then a comparatively simple mechanism can account for the observed behaviour. If, however, the critical variable is the prey density H , then the mechanism will have to be much more complicated with, in effect, two separate switches to control a single response. We conclude that H/P is far more likely to be the correct choice.

The curves in Figs 2 and 3 were obtained by fitting cubic splines to the data of Dent *et al.*³ and we must ask how sensitive our results are to small variations in the shapes of these curves. It is clear that in Fig. 2 no minor change in the curves nor difference of an hour or two in the location of the peaks and troughs of amoebal biovolume can alter the configuration of the jumps. The catastrophe set will remain qualitatively the same and neither the Maxwell nor perfect delay conventions will be

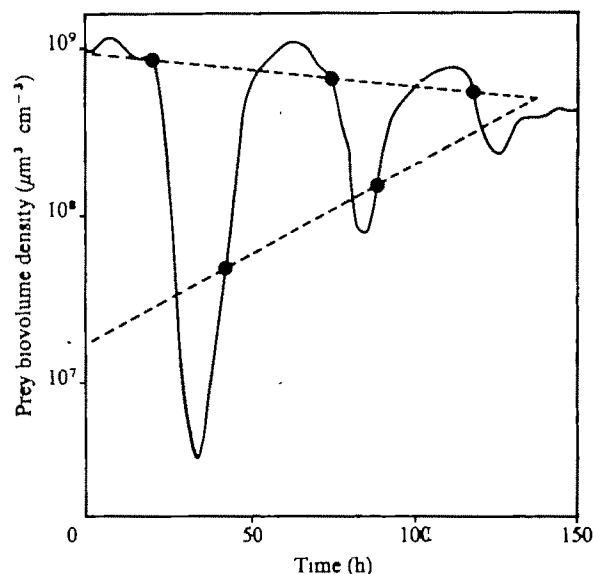


Fig. 3 The same as Fig. 2 but with prey-predator ratio as ordinate.

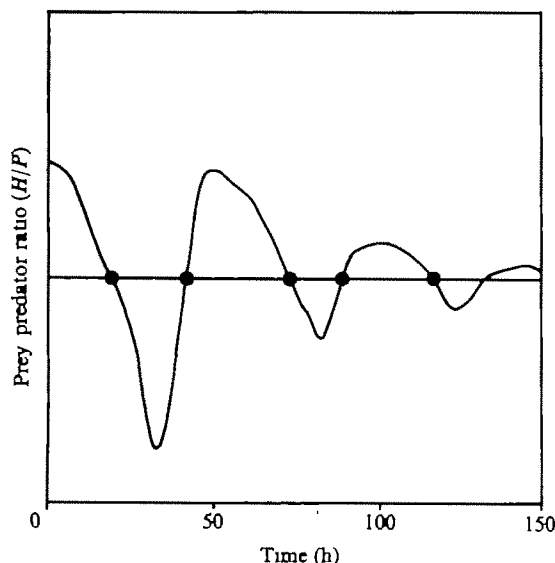
applicable. In the case of Fig. 3, however, we cannot rule out the possibility that the dynamic follows the perfect delay convention with a very narrow cusp. This would matter if we were trying to guess the actual mechanism, but it does not affect our argument concerning the relative simplicity of the possible mechanisms. Our conclusions thus do not depend on the precise shape of the curves.

If, as we believe, the rate of growth of amoebae is controlled by H/P , there must be some mechanism by which the amoebae can estimate this ratio. It has been suggested² that folic acid, which is secreted by *E. coli* (and by soil bacteria as well) may serve as an attractant for food-seeking amoebae. If folic acid molecules are modified on contact with amoebal receptor sites, then the concentration will depend on the ratio of prey to predator densities. We are doing experiments to determine whether the folic acid concentration governs the behaviour of the amoebae.

We disagree with Zahler and Sussman⁴ that "catastrophe theory is a blind alley". By applying it we have discovered what seem to be the correct critical variables in our system. The theory made us concentrate on the amoebal specific growth rate, and ask how this could be affected. The need for two control variables led us to consider culture age as an explicit variable, even though most equations used in population dynamics are autonomous. This provided a link between the early stage of the experiment described here and the observation² that after about 200 h there is an apparently spontaneous change in the population densities of both organisms. Finally, catastrophe theory has provided strong evidence that it is the ratio of biovolume densities to which the amoebae respond, and this has suggested further experiments.

We expect that the way in which we have used catastrophe theory to decide between alternative choices of critical variables will find other applications in the study of complex systems. It does not require data as accurate as would be necessary to fit a model based on differential equations. It requires no definite hypothesis concerning the mechanism of the system and it leads to conclusions that are comparatively robust, for they do not stand or fall with one particular choice of mechanism or *ad hoc* equation. We do not claim that we are somehow obtaining more information from our analysis than was contained in the data—no mathematical technique can accomplish this. What catastrophe theory can do, however, is enable us to answer a limited number of questions about a system on the basis of much less information than would be needed for a complete description. Much of the power of the theory lies in the fact that this limited

Fig. 2 Biovolume density of *E. coli*, with the catastrophe set indicated. The points at which the sudden changes in λ occurred are marked by solid circles. Data are based on ref. 3.



number of questions so often turns out to include the most interesting ones.

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Asynchronous sexual development determines the breeding system in field beans

THE breeding system of varieties of field bean (*Vicia faba* minor L.) has long been known to be intermediate between allogamy and autogamy, with approximately 1/3 of the seeds set as a result of cross-fertilisation and 2/3 by self-fertilisation¹. Cross-fertilisation is due to bees visiting open flowers. Most inbred lines show poor seed set unless their flowers are visited by bees or artificially manipulated (tripped) and are therefore called autosterile; a few lines which do not require tripping are called autofertile^{2–3}. Irrespective of variety, so far as is known, the field bean does not possess a self-incompatibility system for regulating fertilisation^{4–6}; also anther dehiscence always precedes flower opening by one or two days. Why then does the temporal advantage of self pollen over cross pollen not lead to obligate autogamy under field conditions? In an attempt to answer this question, it has been suggested that a spatial separation between discharged pollen and the stigma is normally present, but is abolished by bee visits which simultaneously bring about pollen–stigma contact and chance cross-pollination. According to this hypothesis, autosterile lines have a serious impediment to pollen–stigma contact, whereas, due to a different floral architecture, autofertile lines do not⁷, although intermediate floral structures also occur. Here we report results of a scanning electron microscopic (SEM) study of the stigmas of an autosterile line (T2) and an autofertile line (T51), which lead to an alternative explanation for autosterility and autofertility.

After freezing open flowers in vapourised liquid nitrogen at –110 °C the petals were removed without causing disturbance to the stigma region. Flowers of the autosterile line T2 examined

Fig. 1 Scanning electron micrographs of uncoated *Vicia faba* stigmas at 7.5 kV × 160. *a*, Line T2 after flower opening stage showing stigma surface covered with pollen grains: flower frozen in vapourised liquid nitrogen and dissected without stigma disturbance. *b*, Line T2 emasculated before anther dehiscence showing exudate on stigma surface 18 h after treatment (c.f. Fig. 2*d*, 2*e* for controls).

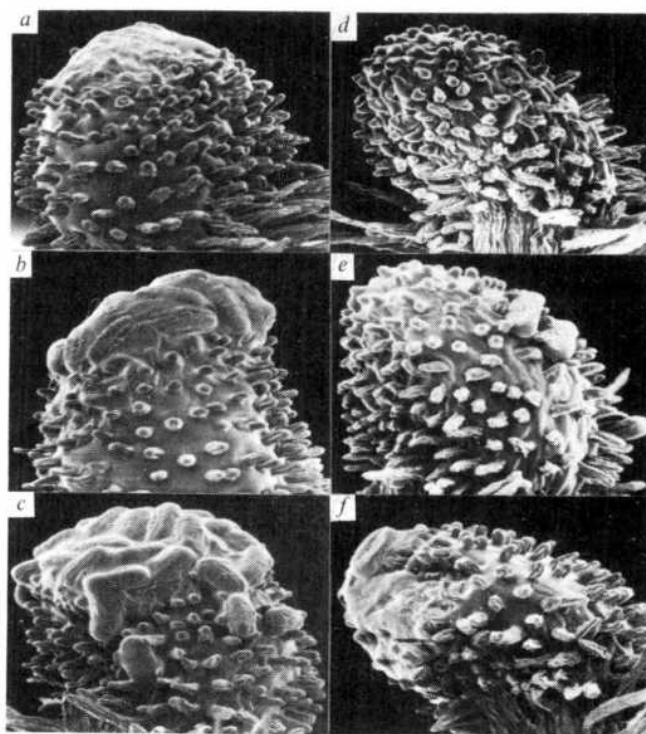
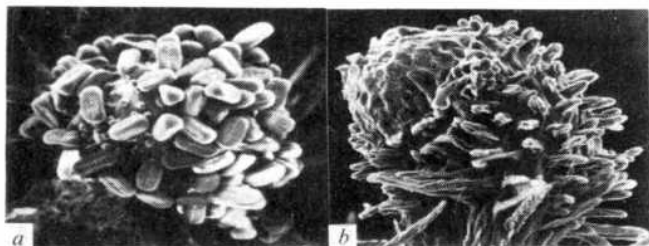


Fig. 2 Scanning electron micrographs of uncoated *Vicia faba* stigmas taken at 7.5 kV × 160, showing exudate appearance during flower development of line T51 (*a–c*) and line T2 (*d–f*). Flowers were taken from the 4th to 8th flowering node at comparable developmental stages: before anther dehiscence (*a*, *d*); after anther dehiscence but before flower opening (*b*, *e*); after flower opening (*c*, *f*).

in this way were found always to have stigmas covered with pollen grains (Fig. 1*a*), showing that autosterility is not due to a spatial separation of stigma and self pollen grains in the undisturbed flowers. Autofertile lines also showed abundant pollen. Stigma surfaces were then examined by SEM after ungerminated pollen grains had been removed from the stigma surfaces by briefly rinsing in distilled water. This investigation showed differences between lines T2 and T51 with regard to the amount and time of appearance of exudate on the receptive areas of the stigma tip (Fig. 2*a–f*). In the autofertile line T51, exudate was present before anther dehiscence (Fig. 2*a*). After dehiscence but before flower opening, self pollen was seen entrapped in an increased quantity of exudate (Fig. 2*b*). Even more exudate was apparent on the stigmas after the flowers opened (Fig. 2*c*). In the autosterile line T2, however, there was no exudate before anther dehiscence (Fig. 2*d*) or before flower opening (Fig. 2*e*), although small amounts of exudate were found on stigmas from open flowers (Fig. 2*f*).

In angiosperm species, including *Vicia faba*, in which a wet stigma is characteristic, it has been suggested that stigma receptivity is signalled by an accumulation of exudate on the stigma surface⁸. In autofertile line T51 and in some other lines (data not shown) where exudate appears and anthers dehiscence before flower opening, the probability of self-fertilisation might be expected to increase. This has been found to be so in at least one instance, where under field conditions⁹ an autofertile line showed much lower outcrossing than did corresponding autosterile lines.

In artificial field bean pollination, manual tripping of open flowers is a recommended practice for increasing seed set in autosterile lines¹⁰. Consequently, we examined the influence of tripping and emasculating on exudation and found, as expected, increased stigma exudate after both treatments (see Fig. 1*b*).

On the basis of these findings the so-called autofertile lines can be classified as protogynous and autosterile lines as protandrous. It seems that the timing of stigma receptivity, as manifested by exudation, acts as a critical control in field bean

pollination. This role of the stigma in actively regulating the breeding system may possibly apply to other bee-pollinated papilionaceous species.

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Does colour provide an input to human motion perception?

A PUZZLING feature of the visual system of primates is the occurrence of a multiplicity of separate 'maps' of the visual environment, each of which has its own independent set of afferent connections. Perhaps these maps represent an early stage in the analysis of visual information by the brain. Electrophysiological studies have shown that depth is analysed in V_2 (area 18) (ref. 1), that colour-coded cells occur mainly in V_4 and that motion in the visual field is analysed in the posterior bank of the superior temporal sulcus² (Fig. 1). We report here an experiment which suggests that colour and movement are handled separately in the visual system and that information defined by wavelength cannot be processed by the brain's motion-detecting mechanisms. This finding is consistent with Zeki's demonstration that "cells of the movement area are not concerned with colour"^{2,3}.

Stimuli used were based on Julesz' random-dot stereograms⁴ (Fig. 2). The members of the pair of random-dot arrays are identical except that all the dots of a central square-shaped region are horizontally shifted to an equal extent in one display in relation to the other. On monocular inspection, only random dots are seen for either display, but when fused binocularly, the central shifted region stands out in depth from the rest of the dots, as a square having a sharp border; this may be described as an illusory contour, as it is not defined by the (irregular) dots of the displaced region.

Lu and Fender⁵ found that when such a display is presented with coloured dots against a contrasting colour background, stereoscopic depth is lost when the dots and the background have equal luminance, although the dots are clearly seen by colour contrast at isoluminance.

The use of random-dot stereograms has not been limited to stereoscopic experiments. In the present experiment, random-dot displays are not presented to give stereoscopic depth; they are optically superimposed, and are exposed alternately at a rate which gives apparent motion of the central displaced region, which then appears as a square oscillating horizontally^{4,6}. The square has (very much as for stereoscopic presentation) a clearly defined illusory border. Neither the border nor the motion are visible without alternation of the pair of dot displays. The

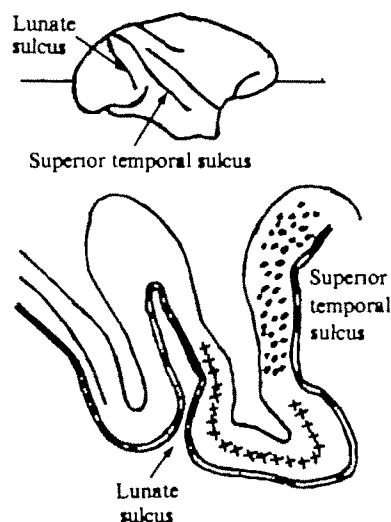


Fig. 1 The lower diagram shows a horizontal section of the brain of a rhesus monkey taken at the level indicated in the upper diagram to show regions of the prefrontal cortex that are specialised for colour (marked by crosses) and motion (stippled) (adapted from Zeki²).

appearance of the border can be used as an independent and unambiguous criterion of motion perception⁷.

We used these patterns to study the role of colour as an input to human motion perception. Each dot in the original pattern (Fig. 2) is defined by a stepwise change in intensity (that is, black dots on a white background). Our strategy was to convert these intensity gradients into gradients of 'pure' wavelength; that is, we replaced all black-white borders with red-green borders using an isoluminance projection system⁸. The intensity of the green areas was then varied continuously over a wide range so that at least at some setting the intensities of red and green must have been precisely matched.

Isoluminance, with exact registration of the colour areas to prevent brightness contours, was obtained by projecting a special colour transparency with combined epi and dia projection through a single lens⁸. Two light sources were independently controlled for intensity, the epi source, which illuminates the upper coloured (red) surface of the opaque regions of the transparency, and the dia source, which projects by means of a colour filter (green) through the transparent regions. The transparency may be photographically produced silk screen prints; or 3M colour-key contact imaging material (red opaque negative), as used here. The format was 60 mm².

An identical pair of these projectors allowed a Julesz pair of transparencies to be superimposed, and projected alternately with a pair of electrically synchronised rotating sector-disk shutters. 'Key-stoning' distortion was avoided by placing one projector behind and the other in front of a translucent screen.

Fig. 2 A Julesz random-dot stereogram (see text for explanation). The two patterns were optically superimposed and exposed alternately to produce apparent motion.



The projected display was 37 cm² viewed from about 1 m, subtending 65°. The dot elements were 2 cm, subtending 3°, which was at least two orders above visual resolution at isoluminance. The screen luminance at isoluminance match was about 0.34 cd m⁻². Similar results were obtained at higher intensities (up to 34 cd m⁻²).

Four volunteers were used and two of them were unaware of the purpose of the experiment. Each subject began by deliberately introducing a large brightness difference between the red and green areas. At a frequency of about 100; 50; 100 (stimulus duration 100 ms; interstimulus interval or ISI 50 ms), a square with well-defined edges could be seen oscillating back and forth horizontally. Using the luminance control knob the intensity of the green dots was then varied continuously over a wide range. The subject was asked to record what he saw as he rotated the knob.

As the green background luminance was gradually reduced from a high value to near isoluminance, all subjects reported disappearance of the square. The square then reappeared when the luminance of the green was reduced to a level below the 'isoluminance' point. The disappearance of the square was quite sudden, and reported spontaneously by all four subjects.

Table 1 Critical range of luminance of green dots for each of four subjects

Subject	Luminance range (cd m ⁻²) over which square was invisible	Luminance range (cd m ⁻²) at which motion was invisible
1	4.42	6.84
2	4.32	6.84
3	4.73	7.68
4	3.49	7.68

Each entry represents the mean value of four readings.

Table 1 shows the 'critical range' of intensities over which the square disappeared for each of the four subjects. For all subjects: (1) the square disappeared slightly earlier than motion and was completely invisible over a narrow range of about 58% which was fairly constant for each subject, (2) the luminance setting for motion disappearance was remarkably constant among subjects. At this setting, three of the subjects reported that they could still see a slight 'shimmering' but none of them could detect any motion.

Having found the critical range, we varied other parameters to see if the square could be restored. Increasing the frequency of alternation did not make any difference. On lowering the frequency to about half the original rate, some of the dot elements could be seen moving, but the direction of motion was ambiguous and the edge defining the square remained invisible. Neither the square nor unambiguous motion could be restored at any frequency. We also tried doubling or halving the size of dot elements and/or the spatial displacement of the inner square, but these proved equally ineffective.

It is important to note that at the setting at which motion disappeared, the individual dots could be discerned just as clearly as when luminance contrast was present. Conversely, even gross blurring of the pattern (by defocusing the projectors) did not destroy the square as long as brightness differences were preserved.

In a control situation we used light red-dark red dot patterns instead of red-green. The intensity of one of the reds was then varied continuously. All four subjects reported that the disappearance of the square (and of motion) coincided almost exactly with the actual disappearance of the dots at isoluminance. The critical range for the disappearance of the square and of motion was thus much narrower than that measured using red-green dots (Table 1). Thus, colour cues seem to have, if anything, a slightly detrimental effect on the perception of coherent apparent motion.

The disappearance of motion at isoluminance is exactly analogous to Lu and Fender's⁵ discovery that stereopsis disappears at isoluminance in stationary random-dot stereograms. 'Classical' stereopsis, using monocularly visible line targets, apparently does not break down completely at isoluminance⁹, and we now find that this is also true of apparent motion. If two simple line targets are alternated instead of random-dot patterns, motion does not disappear at isoluminance.

As in the case of stereopsis⁹, random-dot targets may be processed differently from simple line targets. The former must involve cooperative interactions between large populations of cells tuned to either the same disparity^{4,10,11} or even (in the case of motion detectors) the same direction and velocity. Our findings may imply that at isoluminance cooperative facilitation is not possible between colour contrast-sensitive motion detectors. Alternatively, the relevant motion detectors may be insensitive to wavelength, which is consistent with physiological evidence³, and especially with the observation that monkey colour-opponent neurones give sustained responses whereas broad-band non-opponent neurones respond transiently¹²⁻¹⁴.

Regardless of our interpretation, it is clear from our findings that colour and motion are handled separately by the human visual system and that colour provides only a weak 'cue', at best, to movement perception.

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Suppression of oestrogen-induced LH surges by social subordination in talapoin monkeys

It is axiomatic that the social structure of a group of primates has a pervasive effect on each individual's behaviour. Thus, many monkeys form dominance hierarchies, based on the successful outcome of aggressive interactions, and the more subordinate animals show characteristic behavioural traits^{1,2}. In particular, the social hierarchy has marked effects on reproductive behaviour, especially in talapoin monkeys, where sexual interactions tend to be the prerogative of the dominant males³. Subordinate male rhesus monkeys have limited access to sexually attractive females and produce fewer offspring⁴, and in the few species that have been carefully examined, subordinate females, although apparently receiving sexual attention, are, nevertheless, less fertile than would be expected⁵⁻⁷. Recently, it has become clear that the dominance hierarchy affects not only behaviour but also hormone levels. Testosterone is higher in dominant males^{8,9}, cortisol and prolactin are raised in more subordinate animals¹⁰. We report here evidence that subordination can prevent the luteinising hormone (LH) surge which

Table 1 Sexual and aggressive behaviour, plasma cortisol and prolactin in female talapoins

		Dominant female	Subordinate female	<i>z</i>	<i>P</i>
Total aggression received from males	Attacks	45	110	2.099	<0.02
	Threats	11	15	0.54	NS
	Displaces	8	61	3.79	<0.001
Total aggression received from females	Attacks	2	55	3.52	<0.0002
	Threats	0	11	2.28	<0.01
	Displaces	0	216	8.79	<0.0001
Sexual behaviour received	Mounts	339	64	7.31	<0.0001
	Ejaculations	139	6	7.52	<0.0001
Mean plasma prolactin ± s.d. (ng ml ⁻¹ , human standard)		61.4 ± 43.0	125 ± 55.4	<i>t</i> 8.22	<0.001
Mean plasma cortisol (µg ml ⁻¹ ± s.d.)		176.5 ± 43.1	207.6 ± 76.6	2.4	<0.02

Behaviour was based on 1,000 min of observations during five separate two-week periods when females received oestrogen. Plasma samples were collected over a 10-month period during which females received their oestrogen implants. Attack: the aggressor chases its opponent and attempts to grab and bite it. Threat: eyebrows are raised, eyes open wide and the mouth open to varying degrees. Displace: such aggression often occurs when the subordinate monkey is occupying a food hopper or sitting with another monkey. Without necessarily making contact the dominant monkey may cause the subordinate to move away and then occupy the same position. (*z*, Mann-Whitney; *t*, Student's *t*-test). NS, not significant.

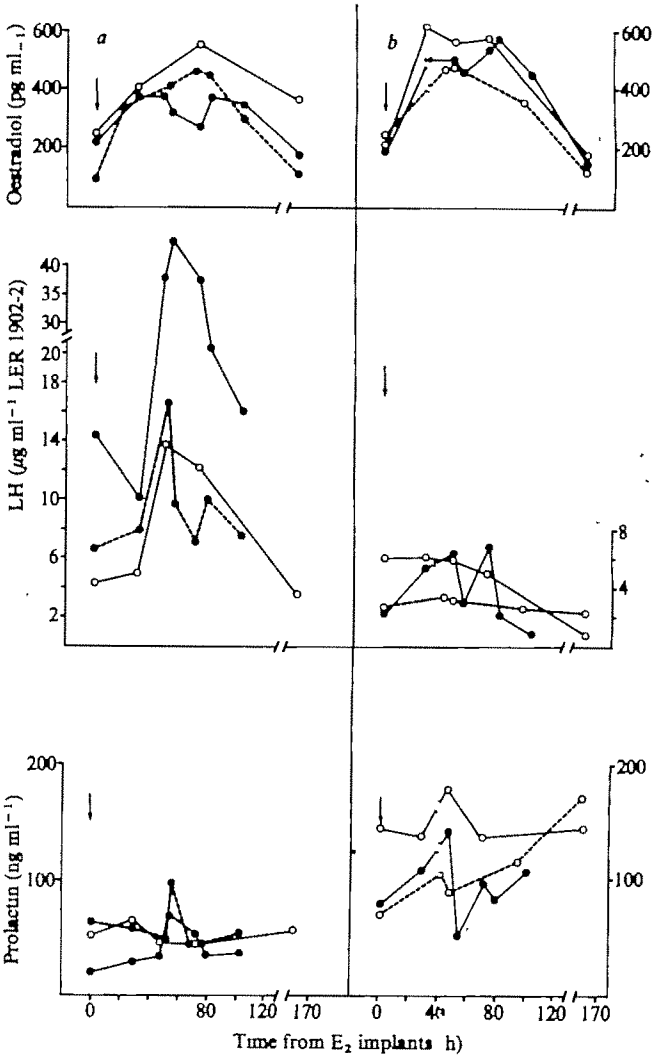
is normally induced by giving oestrogen to female monkeys, thus offering an explanation for reduced fertility and suggesting that this can be induced by behavioural interactions.

A socially living group of four male and four female talapoins was studied in a large cage (11' × 8' × 5') in which they had lived for about one year before the experiments described here. Males and females were denied access to each other apart from for two periods of 50 min d⁻¹, when sexual, aggressive and social behaviour were scored quantitatively from behind a one-way vision screen as described elsewhere¹¹.

The males were intact; females were ovariectomised and were implanted with a single oestrogen-filled capsule (1.5 cm × 3.2 mm × 1.0 mm) producing plasma levels of 100–150 pg ml⁻¹, approximating those occurring in intact females during the follicular phase of the cycle. These were removed and replaced at intervals to ensure maximal sexual skin swelling and female attractiveness. The administration of an oestrogen surge to the hypothalamo-pituitary axis is a well recognised method of inducing discharge of LH in primates¹². An oestrogen surge was produced in the female talapoin by inserting two additional oestrogen-filled capsules and plasma samples were then taken at intervals over the following 5 d. Plasma oestradiol¹³, LH¹⁴ and prolactin¹⁵ were measured by radioimmunoassay and the animals' behaviour continued to be scored twice daily.

Before oestrogen surges were given, the group had been observed for about one year and plasma samples taken twice weekly. During this time, both dominant and subordinate females received the same dose of oestrogen. Both engaged in sexual interactions, although this was more frequently observed in the case of the dominant female (Table 1). Aggressive behaviour was also different, the subordinate female receiving much more than the dominant. Plasma cortisol and prolactin levels were raised in the subordinate compared to the dominant female during the time they lived together (Table 1). The dominant female when given an oestrogen surge on three occasions (each separated by approximately 21 d) always produced an LH surge about 50–70 h later; in no case was this observed in the subordinate female even though oestrogen levels were comparable to those of the dominant animal (Fig. 1). During treatment with additional oestrogen, the behavioural differences between the two females were again observed, the dominant female receiving more ejaculations (18 mounts and 15 ejaculations in 10 h observation) than the subordinate (14 mounts, 0 ejaculations in 10 h observation). No aggressive interaction was directed towards her although a considerable amount was received by the subordinate (10 attacks, 4 threats,

Fig. 1 Plasma LH and prolactin levels in dominant (a) and subordinate (b) female talapoin monkeys when given extra oestradiol (E₂) implants (indicated by arrows) over a five-day period. The subordinate female has significantly higher prolactin levels and unlike the dominant female does not respond with an LH surge



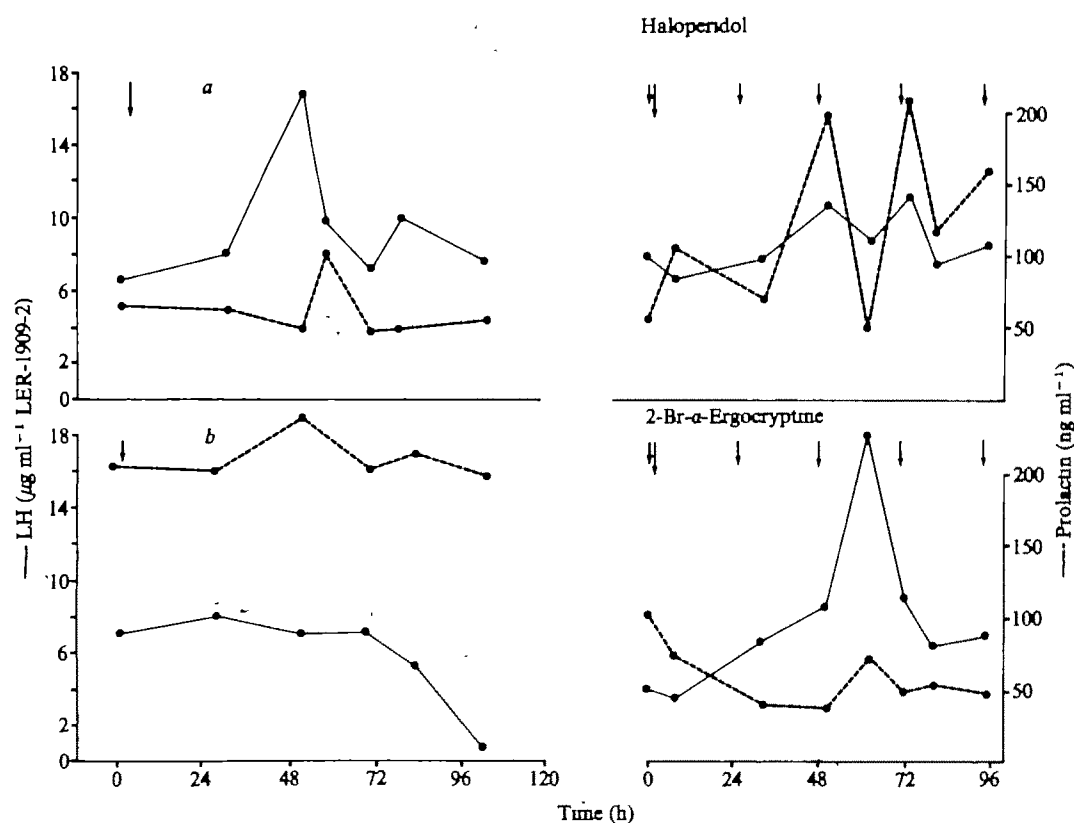


Fig. 2 Effects of suppressing or elevating prolactin on the oestrogen (indicated by large arrow) induced LH surge. The subordinate female (b) receives bromocryptine treatment by daily injection (indicated by small arrows) which lowers plasma prolactin and permits an LH surge. The LH surge is blocked when plasma prolactin is elevated by haloperidol treatment in the dominant female (a).

14 displaces). Plasma prolactin continued to be significantly higher in the subordinate female (mean $115.7 \pm 35.3 \text{ ng ml}^{-1}$) than the dominant female ($50.8 \pm 17.0 \text{ ng ml}^{-1}$), $t = 7.15$, $P < 0.0001$) during the oestrogen challenge (Fig. 1). The subordinate female was subsequently treated with a dopamine agonist, bromocryptine, a procedure which effectively lowers prolactin in humans and can restore ovulation^{16,17}. This resulted in the reduction of plasma prolactin levels, and when challenged again with two oestrogen-filled capsules during bromocryptine treatment, an LH surge occurred which resembled that formerly seen in the dominant female. Conversely, the prolactin levels in the dominant female were raised by giving her the dopamine receptor blocker, haloperidol. This both raised her prolactin level and rendered the oestrogen challenge ineffective, no additional LH being released (Fig. 2). (It is also well recognised in humans that high prolactin levels are associated with amenorrhoea and failure to ovulate¹⁶.)

As it was possible that the raised prolactin levels in the subordinate female might be due to factors other than her social environment, she was removed from the group and housed singly. Eight weeks later, her prolactin levels had fallen to those comparable with the more dominant animals, and the oestrogen challenge now induced an LH surge without the need for bromocryptine treatment.

Our results show that behavioural interactions can so alter the female primate's neuroendocrine system as to render it unlikely that she could conceive. Thus, reduced fertility observed in certain subordinate female primates in the wild, and that observed in humans during stressful situations¹⁸, could be related to the hormonal changes we describe here. We cannot be sure that the effect of the drugs on LH secretion was due to the consequent changes in prolactin, for dopamine is also implicated directly in the release of LH¹⁹. It is certainly possible that high levels of prolactin, however produced, are inhibitory to ovulation and hence fertility. Furthermore, we can speculate that the syndrome of hyperprolactinaemia in women could be induced or aggravated by behavioural mechanisms. Of course, these would not necessarily involve overt aggression as described in the talapoin monkeys, but whatever factors threaten the social stability of the individuals concerned, and the way in which they

react to this, would provide a more likely parallel for our own species. While not wishing to imply that all subordinate monkeys are necessarily infertile, such a mechanism, if it is physiologically active, may also be a way whereby subordinate females would contribute less to the breeding of a group than might be expected by simply observing their overt sexual behaviour.

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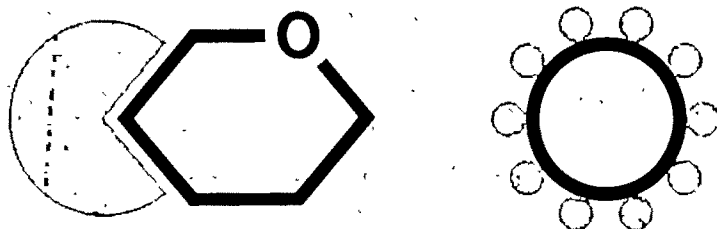
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Lysosomal and mitochondrial heat labile enzymes in ageing human fibroblasts

ONE of the characteristic changes associated with the ageing of human fibroblasts in cell culture is the presence of increasing amounts of altered molecules¹⁻³. Such alterations have been detected for at least three cytoplasmic enzymes: glucose-6-phosphate dehydrogenase (G6PD), 6-phosphogluconate dehydrogenase and hypoxanthine-guanine-phosphoribosyl-transferase. For each enzyme, the proportion of molecules which are modified reaches as much as 25% in cells of the last generations. The origin of these alterations is still only an hypothesis. Some authors¹, in agreement with Orgel's error catastrophe theory⁴, suggest that they are the result of a faulty synthesising machinery; others⁵⁻⁷, however, suggest that they are due to a post-translational effect. We present here a study on the heat lability of some lysosomal and mitochondrial enzymes to test whether the alterations also affect proteins present in these subcellular entities. We compare the results with the predictions of the above hypotheses, and suggest that they probably are best explained in terms of a post-translational modification.

Experiments were carried out on normal human fibroblasts (WI-38). Using Hayflick's method⁸, these cells were cultured and the population was split 1:4 every week. Cells from confluent cultures were collected, homogenised and heated to high temperatures so as to control the denaturation rates of the enzymes (Fig. 1 and Table 1). Five enzymes were tested: *N*-

acetyl- β -D-glucosaminidase (NA β Glu), α -D-glucosidase (α Glu) and *N*-acetyl- α -D-galactosaminidase (NA α Gal), all located in the lysosomes, and sulphite cytochrome *c* reductase and G6PD, located in the mitochondria and cytoplasm, respectively. For NA β Glu, only isoenzyme B was considered. Isoenzyme A, which had first been inactivated by incubating the homogenate for 60 min at 37 °C in an imidazole-HCl buffer at pH 7 (ref. 11), was responsible for 10% of the enzyme activity in both young and old cells. The protein concentration of the homogenate was adjusted to obtain accurate assays on the enzymes (Table 1). The slight variations in the protein content of the homogenate did not affect the denaturation rates of the enzymes but we added albumin to serve as a protective agent when high dilutions of this homogenate were required. On the other hand, temperature and pH controls were crucial in such experiments. Two cell populations from the same line were compared: those from passages 22-25 and those from passages 42-45, which had completed 46-53% and 89-96% of their maximum lifespan, respectively. The maximum lifespan was observed on cells of the same lineage.

The experimental points presented in Fig. 1 were subjected to a factorial two-way analysis of the variance and showed a non-significant time-age interaction except for a significant interaction with G6PD ($P < 0.01$). When this enzyme was tested in the old cells, the deviation of the curve from linearity was significant ($P < 0.05$) and the experimental values fitted best by superposing two decreasing exponentials; the first one, with the steeper slope, represented the denaturation rate of the heat-labile G6PD, and the second one, that of the unaltered enzyme. The latter was responsible for 77% of the enzyme activity. Thus, in the analysis made in our laboratory, only the G6PD was altered in the old cells. The experiments were

Fig. 1 Inactivation curves of enzymes from young and old WI-38 fibroblasts. Confluent cultures were rinsed with phosphate buffered saline (PBS) and the cells were collected with a rubber policeman. They were then washed twice with 0.25 M sucrose solution and homogenised by 20 strokes of the tight pestle of a Dounce homogeniser (Kontes). For the assay of sulphite cytochrome *c* reductase, the cells were disrupted with an ultraturax. The homogenates were heated in the incubation medium to the temperatures critical for the denaturation of the enzymes. At given times, samples were removed and tested for their residual enzyme activity. The experimental conditions are summarised in Table 1. Enzyme assays were introduced following methods already published but with slight modifications, that is, NA β Glu, α Glu and NA α Gal were tested with umbelliferyl derivatives⁹ at pH 3.5, 4.5 and 4, respectively; sulphite cytochrome *c* reductase as originally described at pH 8.5 (ref. 10); the preparation of the extract and the assay of the G6PD were carried out following the method of Holliday and Tarrant¹. Results were expressed

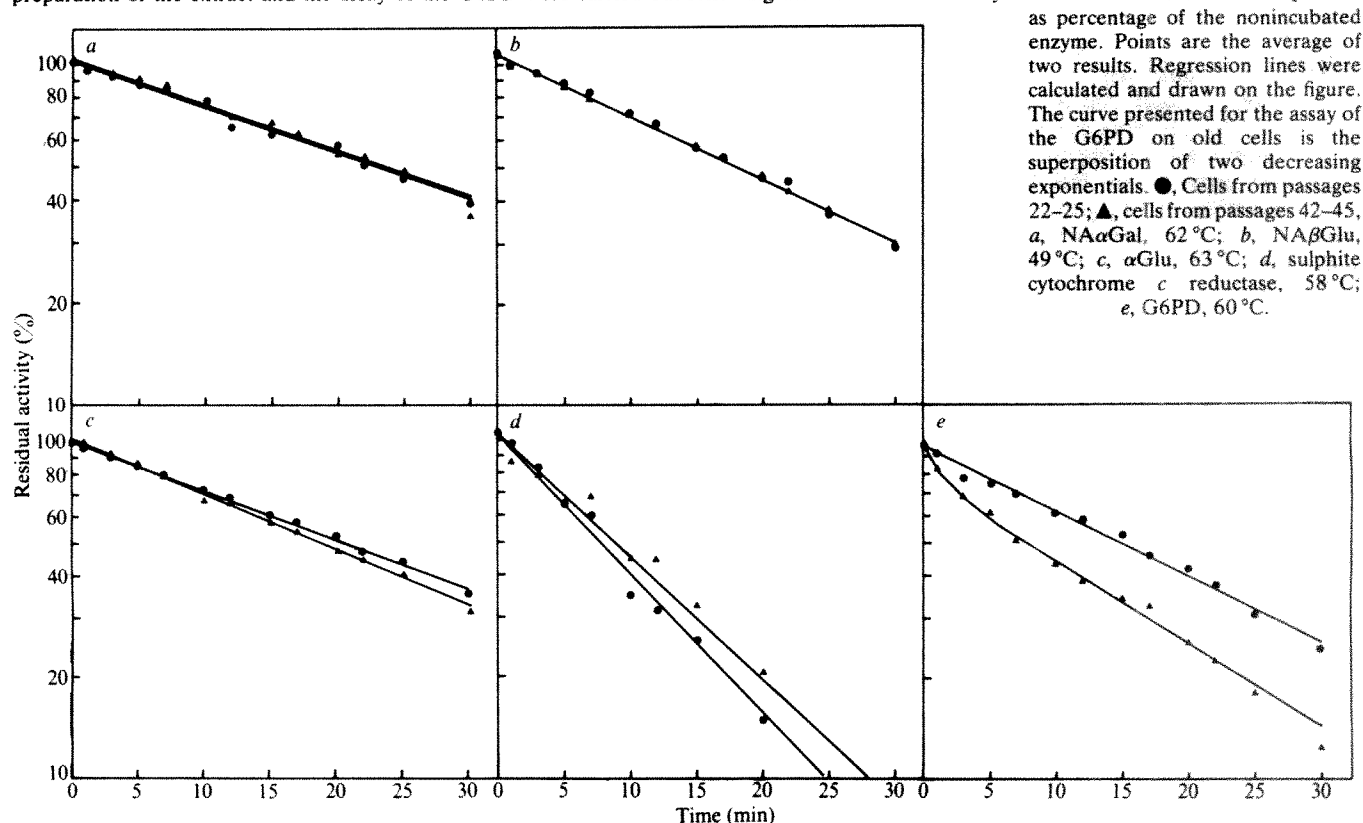


Table 1 Experimental conditions for the inactivation curves

Enzyme tested	Range of the protein concentration of the homogenate (mg ml ⁻¹)	Buffer	pH	Other components	Temperature of incubation (°C)
N-acetyl- α -D-galactosaminidase	1-1.5	5 mM acetate	4	Albumin 1 mg ml ⁻¹	62
N-acetyl- β -D-glucosaminidase	0.003-0.005	50 mM citrate	3.5	Albumin 1 mg ml ⁻¹	49
α -D-glucosidase	0.1-0.15	10 mM acetate	4.5	Albumin 1 mg ml ⁻¹	63
Sulphite cytochrome c reductase	5-8	3 mM imidazole-HCl	8.5	Sucrose 0.25 M	58
Glucose-6-phosphate dehydrogenase	1.5-2	50 mM Tris-HCl	8	Mercaptoethanol 1 mM EDTA 1 mM α -amino-n-caproic acid 1 mM NADP 1 mM	60

repeated at least twice, as described above, and once again at a lower temperature (2 °C below that indicated); within a range of 2-5% no alteration could be detected for the lysosomal and mitochondrial enzymes.

Holliday and Tarrant¹ showed that some cytoplasmic enzymes are modified during the ageing of human MRC 5 fibroblasts in culture. We confirmed these results with WI-38 cells and found that the percentage of heat-labile G6PD observed in old populations is similar in both cell strains. To explain the appearance of altered enzymes in old cells, Orgel's error catastrophe theory of ageing¹² was proposed¹. This theory suggests that all the enzyme molecules would be modified, as they are synthesised by faulty machinery. However, we show that at least four enzymes are not significantly modified during the ageing process. One explanation for the fact that modifications would not be detectable in these molecules is that the altered enzymes would be completely inactive. This eventuality is rather improbable, for a misincorporation of amino acids produces considerable heterogeneity in the loss of enzyme activity¹³. The hypothesis of a post-translational modification explains more easily how alterations occur in only some enzymes. Several results have been published supporting this explanation¹⁴⁻¹⁸, but discussion on the subject remains open¹⁹⁻²¹. The presence of unaltered enzymes in lysosomes and mitochondria, apart from the modified cytoplasmic enzymes, also suggests that the alteration could be specific to certain cellular compartments. However, the analysis of many more enzymes would be necessary before we could generalise this hypothesis. Note also that not all the cytoplasmic enzymes are modified, for phosphoglucose isomerase was found to be unaltered in old cell cultures²². In conclusion, the fact that four enzymes located in lysosomes and mitochondria are not modified during the ageing of cell cultures suggests that the error catastrophe theory is not a satisfactory explanation for the presence of such alterations in some cytoplasmic enzymes.

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Initiation of chemical carcinogenesis requires cell proliferation

CELL proliferation has often been implicated in the development of cancer with chemicals¹⁻³. Supportive evidence for this is the observation that several carcinogens that normally do not induce liver cancer in intact adult animals, especially with a single dose, become carcinogenic if administered as a single injection after partial hepatectomy (PH)¹. In these conditions, it is thought that PH may act during initiation, presumably by fixation of some carcinogen-induced DNA damage through replication of the altered DNA. However, because carcinogenesis is a multi-step process, often involving the appearance of several new cell populations between the initial target cells and the ultimate cancer^{4,5}, it is possible that the regenerative response of liver following PH could have a major effect on one or more steps subsequent to initiation. Thus, the use of a very late end-point (cancer) makes it difficult, if not impossible, to relate with any accuracy some very early event to any specific step in the carcinogenic process. Solt and Farber⁶ have recently developed a new approach to the sequential analysis of carcinogenesis *in vivo* that delineates the first few steps in the process and is a quantitative assay for initiation of liver cancer. We have used this model to investigate whether cell replication exerts its first effect on initiation or on some later step in the carcinogenic process. And if initiation is at least one site of action, when in the regenerative cell cycle is a carcinogen most effective and what biochemical events might be involved at this phase of the cell cycle?

The assay⁶ is based on the hypothesis that an early, if not the first, cell is resistant to the inhibitory effect of carcinogens on cell proliferation. It consists of the measurement of the number of foci of carcinogen-altered hepatocytes that are capable of responding to a stimulus for cell proliferation in the presence of a strong inhibitor of normal cell proliferation (for example, low dietary level of 2-acetylaminofluorene, 2-AAF). The combined dietary 2-AAF plus PH creates a strong selection pressure for

the growth of carcinogen-induced putative initiated cells, but does not by itself induce such changes in the experimental conditions used^{6,7}. The foci of altered cells can readily be identified either grossly or with the histochemical use of γ -glutamyl transpeptidase (γ -GT) activity, an enzyme that is a marker for pre-neoplastic liver lesions in these conditions⁷⁻¹⁰. In addition, with a single dose of at least one chemical carcinogen, diethylnitrosamine (DEN), as the initiating carcinogen and with this model, hepatocellular carcinoma develops in the majority of rats within 8 months⁷. The cancers are seen to arise within hyperplastic nodules which in turn are derived from foci of altered resistant hepatocytes⁷. For example, with a large initiating dose of DEN, such as 200 mg per kg, approximately 10^3 foci appear in a 5-g liver during selection, of which about 10^2 develop into persistent hyperplastic nodules. One to five hepatocellular carcinomas develop from such nodules.

For this study, a potent carcinogen for many tissues or organs and one not requiring metabolic activation, *N*-methyl-*N*-nitrosourea (MNU), was selected. This compound is not a carcinogen for intact liver despite its extensive methylation of DNA and other liver macromolecules¹¹, but becomes one when administered after partial hepatectomy¹². MNU was administered intraperitoneally (i.p.) to male Fischer rats (F-344). Three hours later, a time at which no further MNU could be detected in the blood or in the liver (E.C. and D.S.R.S., unpublished), the animals were subjected to either 67% partial hepatectomy or sham operation (SO). After a recovery period of 2 weeks, all the rats were exposed to the selection procedure favouring the growth of the putative initiated hepatocytes over the surrounding hepatocytes. This consisted of dietary 2-AAF plus the liver mitogen, α -hexachlorocyclohexane (α -HCH)¹³, as shown in Fig. 1.

The results presented in Table 1 show that foci of resistant phenotypically altered hepatocytes, positive for γ -GT, were seen in rats which received MNU and which were partially hepatectomised. Very few foci were seen in the animals receiving MNU+SO, vehicle+PH or no MNU. A similar pattern of results was obtained using male Wistar rats. Also, when CCl_4 replaced the α -HCH as a stimulus for cell proliferation, significant numbers of γ -GT-positive foci were again seen only in rats receiving MNU plus PH. Recent results in experiments designed to determine the time relationships between the administration of MNU and PH for optimal initiation indicate that MNU is a much more efficient inducer of putative initiated cells when administered 18 h after operation (129 foci cm^{-2}). The degree of initiation falls off rapidly with administration of MNU before 16 h or after 24 h following partial hepatectomy.

Table 1 Effect of partial hepatectomy on the induction of γ -GT-positive foci in the liver by MNU

Treatment	α -HCH		CCl_4
	Fischer	Wistar	Fischer
MNU+PH	$7.4 \pm 2.5(4)$	12.6 12.5	20.3 26.0
MNU+SO	$0.6 \pm 0.2(4)$	0.9 0.3	$0.8 \pm 0.5(4)$
Citrate+PH	$0.2 \pm 0.1(4)$	0.3 0.5	$0.5 \pm 0.3(4)$
MNU, no operation	0.7 0.4	—	—
No MNU, no operation	—	$0.6 \pm 0.2(5)$	—

For scoring purposes, we used the characteristic histochemical staining for γ -GT, as described by Rutenburg *et al.*¹⁸, with which the selected foci stain dark red and the surrounding normal hepatocytes show no staining. γ -GT-positive foci were counted by low power microscopy and the numbers expressed as foci cm^{-2} of section area (mean $\pm$ s.e.m.). The number of animals is in parentheses. All the groups were selected by exposure to 0.02% 2-AAF plus CCl_4 or α -HCH, as detailed in the legend to Fig. 1.

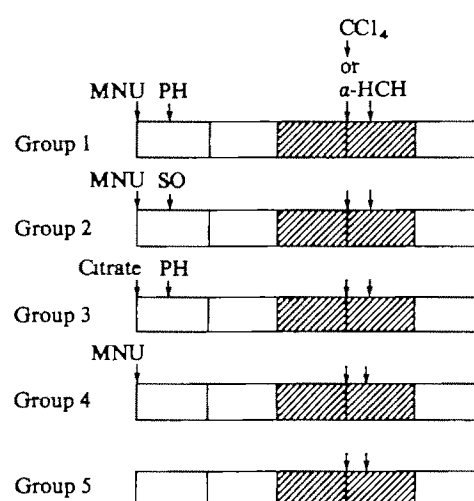


Fig. 1 Adult male Fischer 344 rats (Canadian Breeding Farms and Laboratories) were given a single dose of *N*-methyl-*N*-nitrosourea (60 mg per kg, i.p. in 10 mM citrate buffer pH 6.0). After 3 h they were either submitted to partial hepatectomy (PH) or sham operated (SO). Control groups received MNU and no operation, citrate buffer and PH, or citrate buffer and SO. Two weeks later, all the animals were placed on a diet containing 0.02% 2-AAF in order to create a 'mito-inhibitory' effect, and 1 week after starting the 2-AAF diet they were subjected to a stimulus for liver cell proliferation by force-feeding α -HCH, at a dose of 150 mg per kg dissolved in corn oil, on days 7 and 9 after commencing the 2-AAF diet, or a single dose of CCl_4 (0.25 ml per 100 g in corn oil). After a total period of 14 days on 2-AAF diet, the rats were placed on the stock diet devoid of AAF for 7 days. At the end of this period, they were killed and liver samples were processed for histological and histochemical studies. To monitor the effectiveness of the selection procedure, rats previously initiated with diethylnitrosamine (DEN: 100 mg per kg, i.p.) were also included at the start of the 2-AAF diet. Open bar, 1 week basal diet; hatched bar, 1 week basal diet + 0.02% AAF.

These data clearly indicate that initiation of carcinogenesis with MNU is intimately related to some change in the liver that occurs after removal of two-thirds of the liver.

We have not yet established that the administration of MNU in partially hepatectomised rats in the conditions used in these experiments leads to liver cancer. However, there is considerable circumstantial evidence in support of this. For example, (1) MNU in similar conditions has been found to be carcinogenic for the rat liver in two laboratories^{12,14}; (2) the foci induced by MNU, like those induced by DEN and other liver carcinogens, are positive for γ -GT, a marker enzyme for pre-neoplastic and neoplastic hepatocytes⁸; and (3) the foci are indistinguishable in their appearance and early behaviour from those induced by DEN and the latter have been shown to be precursors for liver cancer⁷.

Although the mechanism by which cell proliferation stimulates the induction of putative pre-neoplastic hepatocytes is not known, replication of carcinogen-damaged DNA before repair offers an attractive mechanism by which the carcinogen-induced DNA damage may be fixed and may thus increase the chances of altered nucleotide sequences in the newly made DNA. In support of this hypothesis, it has been demonstrated that liver DNA containing carcinogen-induced lesions such as those induced by MNU (D.S.R.S., S. Rajalakshmi and P. M. Rao, unpublished observations), dimethylnitrosamine^{15,16} and *N*-hydroxy-2-acetylaminofluorene¹⁷ replicates *in vivo*. Such a phenomenon is not lethal for the cell, since the newly made DNA on the damaged templates is stable *in vivo* for at least one month. Interestingly, several liver carcinogens are necrogenic and induce compensatory liver cell proliferation; some of these induce resistant foci without the need for an exogenous mitogen such as PH (refs 6, 7).

The observation that cell replication seems to be necessary for the induction of putative initiated hepatocytes by MNU suggests that initiation of carcinogenesis may consist of at least two steps: (1) interaction of a carcinogen or its activated derivatives with target molecules, possibly DNA, to induce an appropriate biochemical lesion, and (2) the 'fixation' of the lesion or lesions by a round of cell replication. Such a concept would imply that one or more cellular events closely related to cell replication are an integral part of the initiation process with chemical carcinogens. The similarity between initiation of cancer development with chemicals and transformation of cell cultures with DNA viruses and X rays¹⁹⁻²¹ is noteworthy in that they both require at least one round of cell proliferation. The possibility that the initiating carcinogen is selecting a preformed altered cell rather than inducing a new one *de novo* is thought to be unlikely because of the following observations. (1) No foci of altered cells are selected without previous exposure to the carcinogen. (2) The foci observed are distributed randomly throughout the hepatic parenchyma, despite the striking zonal organisation of the liver. In addition, the demonstration that a carcinogen can permanently alter the progeny of a single cell in culture²² supports the hypothesis that the foci we observe represent a *de novo* induction phenomenon.

The possible role of altered DNA or other macromolecules and of the repair of such damage is now being analysed with this model.

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C-type viral antigen expression in human placenta

THE evidence of C-type virus expression in man includes electron microscopic observation of virus-like particles in 30-50% of a small number of human placentas¹⁻⁴. Because of the limitations of ultrastructural surveys (sample number, specimen size, false negatives) we attempted to detect C-type virus expression in human placenta by indirect immunofluorescence using antisera raised against HEL-12 virus; this is a C-type retrovirus recovered from human embryonic lung cells after *in vitro* cultivation⁵. We demonstrate here C-type virus-related antigen

in 24 of 24 term placentas. The specificity of the immunofluorescence reaction for viral antigen was confirmed by serum absorption tests with purified virus. Preliminary isolation of the reactive antigen from placenta yielded a glycoprotein-rich fraction (β - μ) which blocked binding of anti-HEL-12 virus antiserum with placenta. In addition, antisera raised against β - μ reacted with HEL-12 virus-infected cells as well as with human placenta.

A survey of 24 normal full-term placentas was carried out with anti-HEL-12 virus antiserum. The HEL-12 virus antiserum was prepared by hyperimmunisation of rabbits with gradient-purified HEL-12 virus. The antiserum reacts with HEL-12 virus infected cells of several species but not with uninfected controls⁶. In addition, anti-HEL-12 virus antiserum has not been found to react with normal adult tissues^{6,7}. Comparable samples from the maternal surface of each placenta were quick-frozen in isopentane, usually within 1 h and no longer than 5 h after delivery, and then sectioned. Using a previously described technique⁶, all placentas exhibited the same distinctive pattern of fluorescence with anti-HEL-12 virus antiserum (Fig. 1a). For comparison, a light micrograph of a serial section of the same placenta is presented in Fig. 1b. Arrows indicate the areas in which immunofluorescence was detected. Fluorescence was predominantly associated with cells

Fig. 1 Immunofluorescent reaction in human placenta P28V. *a*, 4 μ m cryostat sections of human placenta (P28V) were washed in PBS and fixed in ether-ethanol (1:1) for 5 min followed by 95% ethanol for 20 min at 25 °C. Sections were then incubated with anti-HEL-12 virus antiserum (1:20) for 30 min in a moist environment at 25 °C, washed three times with PBS, stained with goat-anti-rabbit IgG-FITC conjugate (Behring) for an additional 30 min, and then washed again three times with PBS. Slides were examined with UV illumination using an Olympus BHA fluorescence microscope fitted with an FITC filter and a vertical ultraviolet illuminator. The reaction shown is typical of all of the placentas examined. *b*, A serial section of P28V was fixed in formalin and then stained with haematoxylin and eosin. The syncytiotrophoblast (s), cells of the villous stroma (v) and position of maternal (m) and fetal (f) circulation are marked. The arrows indicate the areas in which immunofluorescence was detected.

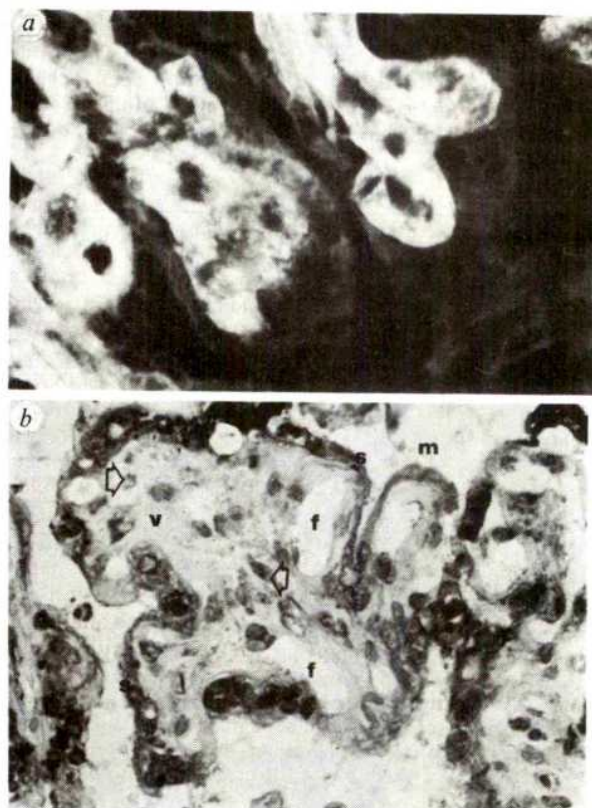


Table 1 Indirect immunofluorescence of human placenta with C-type viral antisera

Antisera (serum dilution)	Absorbent	Concentration (μ g protein)	Immunofluorescence	No. of placentas examined
Premune rabbit (1:20)	—	—	—	24
Rabbit anti-HEL-12 (1:20)	—	—	+	24
Rabbit anti-HEL-12 (1:20)	HEL-12 virus	25	—	5
Rabbit anti-HEL-12 (1:20)	SiSV	25	+	2
Rabbit anti-HEL-12 (1:20)	—	150	trace	2
Rabbit anti-HEL-12 (1:20)	BaEV	25	+	2
Rabbit anti-HEL-12 (1:20)	—	150	trace	2
Rabbit anti-HEL-12 (1:20)	β - μ (3 preparations)	40	—	4
Rabbit anti-HEL-12 (1:20)	RSV	150	+	4
Rabbit anti-HEL-12 (1:20)	Fetal calf serum	1,800	+	2
Rabbit anti-HEL-12 (1:20)	Bovine serum albumin	200	+	2
Rabbit anti-HEL-12 (1:20)	Human serum albumin	200	+	2
Rabbit anti-HEL-12 (1:20)	Haemoglobin	200	+	2
Rabbit anti-HEL-12 (1:20)	Human IgG	200	+	1
Goat-anti-SiSV (1:5)	—	—	+	2
Control goat (1:2)	—	—	—	2
Rabbit-anti-BaEV (1:2)	—	—	—	2

Indirect immunofluorescence of human placenta with C-type viral antisera. Antisera were used in indirect immunofluorescence tests⁶ with frozen sections of human placenta as described in the legend to Fig. 1, with or without previous absorption. Absorption was carried out incubating 50- μ l volumes of anti-HEL-12 virus antiserum (1:20) with 25–1,800 μ g of absorbent protein for 1 h at 25 °C and 18 h at 4 °C. Protein concentration was determined by the method of Lowry²². HEL-12 virus was purified as described⁵. Fetal calf serum (Gibco), human and bovine serum albumins (Pentex), haemoglobin (Sigma) and human IgG (Pentex) were diluted immediately before use. Secondary antisera were goat-anti-rabbit IgG-fluorescein isothiocyanate (FITC) and rabbit-anti-goat IgG-FITC antisera (Behring). Slides were evaluated under code by two independent observers. Each placenta specimen gave the reaction indicated. Specimens were negative (–), positive (+) or trace, a fluorescent reaction which was greatly reduced as compared with positive sample but which could be distinguished from negative samples.

of the villous stroma surrounding vessels carrying fetal blood. No significant fluorescence was found in syncytial trophoblast cells, which are in contact with maternal blood. The fluorescence pattern is consistent with what might be expected if the zone of antigenicity was delineated by the basement membrane between the syncytial trophoblast and the villous stroma. Neither the preimmune serum nor the fluorescein conjugate alone reacted with any placenta. Replicate sections of 10 placentas stained with twofold serial dilutions of antiviral antiserum showed a common endpoint of 1:40, suggesting that they contained a comparable amount of antigen.

Specificity of immunofluorescence was tested by serum absorption. Anti-HEL-12 virus antiserum was absorbed with gradient-purified simian sarcoma virus (SiSV), baboon endogenous virus (BaEV), Rous sarcoma virus (RSV) and HEL-12 virus. Table 1 shows that only HEL-12 virus blocked the reaction completely at the concentrations of virus used. SiSV and BaEV mediated partial absorption, as might be expected, for HEL-12 virus crossreacts with both although they do not crossreact with each other^{5,8,9}. RSV did not block the reaction. Bovine and human serum albumins, immunoglobulin and haemoglobin, at concentrations 10-fold in excess of that needed for HEL-12 virus to absorb, and a 70-fold excess of fetal calf serum, did not block the reaction. Antisera to SiSV and BaEV were tested for reactivity with placenta (Table 1). Both sera detect multiple components of HEL-12 virus as determined by immunoprecipitation and acrylamide gel analysis (J. T. Reynolds, unpublished observations). Anti-SiSV predominantly reacts with the viral envelope whereas anti-BaEV detects a wide range of virion components. Concentrated anti-BaEV antiserum did not react with placenta whereas anti-SiSV antiserum mediated a similar fluorescence pattern to that seen with HEL-12 virus antiserum. The data indicate that anti-HEL-12 virus antiserum reacts specifically with normal human placenta by detecting antigen which crossreacts with a primate C-type virion component. The inability of anti-BaEV antiserum to react with placenta compared with the effectiveness of concentrated BaEV in serum absorption tests with anti-HEL-12 virus antiserum most probably reflects a low antibody titre of the anti-BaEV antiserum for antigenic determinants shared by HEL-12 virus, BaEV and human placenta.

As C-type viral antigens are found at infected cell surfaces¹⁰, a procedure designed to elute cell surface components was used in an attempt to isolate the reactive placenta antigen¹¹. Reactive antigen was monitored during the isolation procedure by the ability of fractions to inhibit immunofluorescence in serum absorption tests. The chorionic membranes and umbilical cords were removed from placenta and the remaining tissue was homogenised and incubated with 3 M KCl for 18 h at 4 °C. The eluted material which was soluble in deionised water was chromatographed on G-100 Sephadex in phosphate-buffered saline (PBS), pH 7.3 (Fig. 2). Protein eluting between 70–80,000 MW, fraction 2, was the most active in serum absorption tests. This fraction was separated by chromatography with concanavalin A-coupled Sepharose (Con A) and a Con A binding fraction (β - μ) was eluted with α -methylmannoside. 40 μ g of this glycoprotein-rich fraction completely blocked the reaction of anti-HEL-12 virus antiserum with placenta (Table 1). In contrast, as 100 μ g of Sephadex fraction 2 was required for comparable inhibition, Con A chromatography selects for material which is increased in specific activity for the reactive

Table 2 Indirect immunofluorescence with anti- β - μ antiserum

Target cell	Infecting virus	Species	Immunofluorescence with antiserum against HEL-12 virus		
			β - μ	HEL-12 virus	Premune sera
HEL-12 p 21	HEL-12	human	+	+	—
A204	—	human	—	—	—
NMF	HEL-12	mink	+	+	—
NMF	—	mink	—	—	—
71AP1	SiSV	marmoset	+	+	—
MFS	—	marmoset	—	—	—
BILN	BaEV	baboon	+	+	—

Rabbit antisera were diluted 1:80 and used in indirect immunofluorescence tests with virus-infected or control cells grown *in vitro*²³. The secondary anti-serum, goat-anti-rabbit IgG-FITC (1:20) did not react with any cell by itself. Positive immunofluorescence with tissue culture cells was granular and apple green and confined to the cytoplasm. Slides were evaluated under code.

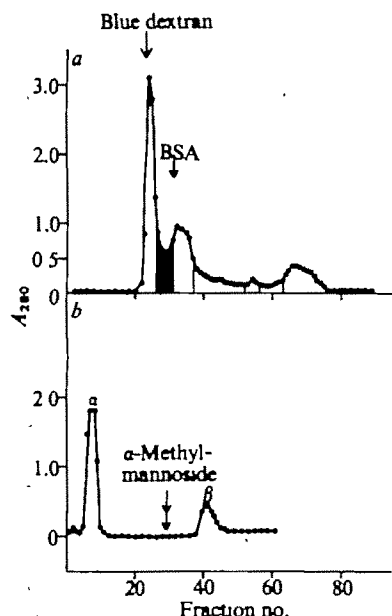


Fig. 2 Fractionation of KCl extract of human placenta. 200–300 g of placenta were obtained by removing the chorionic membrane from freshly delivered human placentas and washing the resultant material with PBS. Three 5-s homogenisations in a Waring blender were followed by treatment for 10 min with 0.85% NH_4Cl at 4 °C and the pellet obtained was treated with PBS containing 3 M KCl for 18 h at 4 °C. The resultant supernatant was extensively dialysed against 0.85% NaCl followed by deionised water. The water-soluble fraction was then concentrated by pressure dialysis and 20–50 mg in 1–1.5 ml was applied to a 150-ml Sephadex G-100 (Pharmacia) column (a). The column was run at a flow rate of 10 ml h^{-1} with PBS, 0.2% NaN_3 , pH 7.0, as the buffer. 2.0-ml fractions were collected and monitored at a wavelength of 280 nm in a Gilford 240 spectrophotometer. The column was calibrated with blue dextran, bovine serum albumin (BSA), and ribonuclease. Fractions were tested in serum absorption tests as described in Table 1. Based on such tests, the fraction shown shaded was selected and applied to a 10-ml column containing Con A-Sepharose (Pharmacia) (b). The column was then rinsed with four column volumes of normal saline, 0.05 M phosphate and 0.2% NaN_3 , pH 7.0, at a rate of 6.0 ml h^{-1} . This was followed by a 10% solution of α -methylmannoside (Sigma). 1.0-ml fractions were collected. The α -methylmannoside eluate was concentrated by pressure dialysis and used for serum absorption tests and immunisation.

placental antigen. β - μ preparations were isolated from three placentas and each preparation blocked the reaction of anti-HEL-12 virus antiserum with specimens from several different placentas.

To substantiate that β - μ contained antigen related to HEL-12 virus, an antiserum was raised in a rabbit against β - μ . Table 2 shows that this anti- β - μ antiserum reacted with virus-infected cells and human placenta but not with uninfected control cells. Preimmune serum did not react with any specimen.

Although we previously reported HEL-12 virus-related antigen and antibody in some tissue from patients with systemic lupus erythematosus⁶⁻⁹, this is the first report of virus-related antigen in all specimens tested of a selected normal human tissue. The placenta may be a privileged site of C-type viral expression in man, as has been suggested for sub-human primates^{2,12}. Factors allowing placenta to be such a privileged site may include hormonal modulation of viral replication^{13,14} and the suspension of some immune mechanisms¹⁵.

The reaction of antiviral antiserum was predominantly associated with cells in contact with fetal blood whereas the rare particles reported in electron microscopic studies¹⁻⁴ were found at the surface of cells bathed by maternal blood. This difference in the location of antigen and maturing particles is of interest in

view of the observation that mammalian cells often contain proviral DNA of non-crossreactive C-type viruses¹⁶. Four unrelated classes of C-type viruses have been described in primates¹⁷⁻¹⁹. In placenta, the virions detected morphologically and the antigen detected by fluorescence may be related. The apparent absence of fluorescence in the syncytiotrophoblast may indicate that viral expression is restricted to small focal zones whereas viral expression in the cells of the villous stroma is widespread. Furthermore, virions in the villous stroma may not yet have been detected. Alternatively, the virions and β - μ antigen may not be antigenically related. In addition, there is evidence that C-type viral antigen may be transiently expressed during gestation^{20,21}.

We have consistently detected the presence of a component of human placenta which crossreacts antigenically with the C-type retrovirus HEL-12. We are able to isolate a fraction from placenta which inhibits this reaction of anti-HEL-12 virus antiserum with placenta and an antiserum prepared to this antigen reacts with placenta as well as with HEL-12 virus-infected cells. We have not determined the site of β - μ synthesis, nor do we know the chronology of its expression during gestation. Experiments are in progress to explore these questions as well as to characterise the complexity of β - μ and determine its presence in fetal tissue.

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Induction of extensive fimbrial branching in the adult rat brain

It is now well known that some central nervous system axons can regenerate following axotomy^{1,2} or sprout in reaction to nearby denervation³. The cholinergic projection from the septal nuclei to the hippocampal formation has demonstrated the ability both to regenerate^{4,5} and sprout^{6,7}. However, the possibility that axons in the mature mammalian brain can be induced to grow in other circumstances has not been investigated. We have examined the ability of the axons of the

fimbria, the fibre bundle which interconnects the septal nuclei and the hippocampal formations, to grow in circumstances other than those used in classical regeneration and sprouting research. The fimbria is particularly suitable for the study of axonal growth in brain as its fibres run as an unbranched bundle, with one surface exposed to the lateral ventricle and thus free from surrounding neuropil. Consequently, it is readily accessible for experimental manipulation. We demonstrate here that fibres of the fimbria in the adult rat brain can be induced to grow in circumstances that neither sever them nor denervate regions to which they normally project.

We used adult albino rats (380–470 g, Sprague-Dawley from Simonsen Labs). All rats, under Nembutal anaesthesia, had their right fimbria exposed at the juncture of the septum, fimbria and anterior hippocampus. A flap of skull was removed for 2 mm on either side of the landmark bregma, and extending from the mid-sagittal suture to the edge of the dorsal surface of the skull. The dura was removed and the cortex, corpus callosum and caudate nucleus overlying and adjacent to the fimbria were aspirated. In some experiments the fimbrial fibres were left untreated, and in other experiments the fimbria was cut with a scalpel or treated for 10 min with a cotton swab containing either 1 μ g of NaCl or 1 μ g of colchicine. The wound was then rinsed with sterile saline and filled with Gelfoam. About two months after surgery animals were anaesthetised and perfused transcardially with a 10% formaldehyde-saline solution (a few animals were perfused with glutaraldehyde for ultrastructural observations). The brains were removed and post-fixed in the perfusion solution before staining and histological analysis. Additional animals were killed at 4 and 11 d after treatment to determine short-term effects of the experimental treatment.

We have previously found that after the fimbria has been cut or crushed the fibres do not regenerate. However, a less traumatic lesion, by freezing the fimbria, produced a most unusual outcome. In some animals, following freeze lesions of the fimbria, a bridge containing nerve fibres and glial and epithelial cells was seen to originate from the fimbria, cross the ventricular space and make contact with the lesioned caudate nucleus⁵ (Fig. 1a, b). These seemed to be regenerating fibres that for some reason could not pass through their normal trajectory and instead followed a path towards the caudate nucleus. However, during experiments on the trophic regulation of synaptogenesis in the mature rat brain we observed that the cotton swab application of colchicine to the fimbria often induced an outgrowth of fimbrial fibres. In some cases these fibres bridged the ventricular space between the fimbria and caudate nucleus. Initially, we suspected that these fibres had regenerated following colchicine-induced damage of the fimbria; however, this interpretation was unlikely in view of morphological evidence that the cotton swab-colchicine application caused very little, if any, fibre destruction (D.G. and C.W.C., in preparation). Accordingly, we proceeded to determine if the application of a cotton swab with salt crystals, or the mere surgical procedure of exposing the fimbria, could induce fimbrial fibres to grow towards the lesioned caudate nucleus.

An outcropping of fibres from the fimbria was seen 30–60 d after cotton swab-NaCl treatment of the fimbria in 13 of 15 cases, and in 5 of these cases there was also an outgrowth of fibres from the lateral septum. These fibres arched out from the fimbria (or septum) but usually curved back and followed a normal trajectory. In material stained for acetylcholinesterase (AChE) a single fibre could be observed to depart from its normal linear trajectory and curve towards the caudate among a tangle of other axons and glial cells. Many axolemmal bulges or varicosities were present in these axons (Fig. 3a). Such varicosities were rarely seen in the normal fimbria. In five of these 15 cases fibres were observed to travel in a dense glial matrix across the lateral ventricle where they contacted the partially damaged caudate (Fig. 2a, b). The marked enrichment of glial cells in the branch was not seen in the normal fimbria.



Fig. 1 a, Photomicrograph of fibre-stained, horizontal section of rat brain 60 d after freezing of fimbrial fibres. Paraffin sections were stained with the Holmes' silver method¹² to demonstrate nerve fibres. The branch is seen to extend from the fimbria (f) to the caudate nucleus (c). Contralaterally, the fimbria and caudate nucleus are intact. Scale bar, 500 μ m. b, Enlarged portion of fimbrial branch to caudate nucleus showing nerve fibres travelling in a mesh of glial cells. Scale bar, 50 μ m.

The fibres in the branch clearly adjoined the caudate neuropil, but it was less clear whether they actually entered it. Ependymal cells lined the rostral surface of the branch and were apparently continuous with the ependymal lining of the fimbria and caudate.

In the initial stages of this apparent growth process, a damming up of AChE-positive material was seen at 4 d post-treatment. By 11 d post-treatment axons could be observed which deviated from their normal pathway and displayed swollen regions along their course (Fig. 3b). These changes were specific to the areas of cotton swab application.

The growth of fimbrial fibres towards the caudate did not seem to be related to the destruction of the fibres, as (1) at short times after treatment, staining for AChE remained normal in the hippocampus (see Fig. 2a) (in contrast to the marked depletion of AChE staining seen after fimbrial lesions⁸); (2) there was little apparent lethal fibre damage in the fimbria as determined by light and electron microscopy; (3) at 4 d after treatment, many normal axons were observed to travel laterally towards the caudate instead of taking the usual longitudinal course along the hippocampal formation; (4) in four cases of complete section of the fimbria no fibre outcroppings were observed.

The conditions for the initiation of growth seemed to depend on two factors. First, some type of mechanical disturbance seemed necessary to elicit growth. In 9 animals whose fimbriae were exposed but left untreated there was only one case where fimbrial fibres grew out towards the caudate. This is in contrast to the cotton swab-NaCl treatment of the fimbria where in 5 of 15 cases fimbrial fibres reached the caudate nucleus. However, in the extreme instance of freezing the fimbrial fibres, axonal growth occurred frequently but involved very few fibres. The most prolific growth occurred with cotton swab applications. It may be that the mechanisms of growth induction are common to all these cases but that in the mere exposure of the fimbria the disturbance is too small, whereas in the freezing of fimbrial

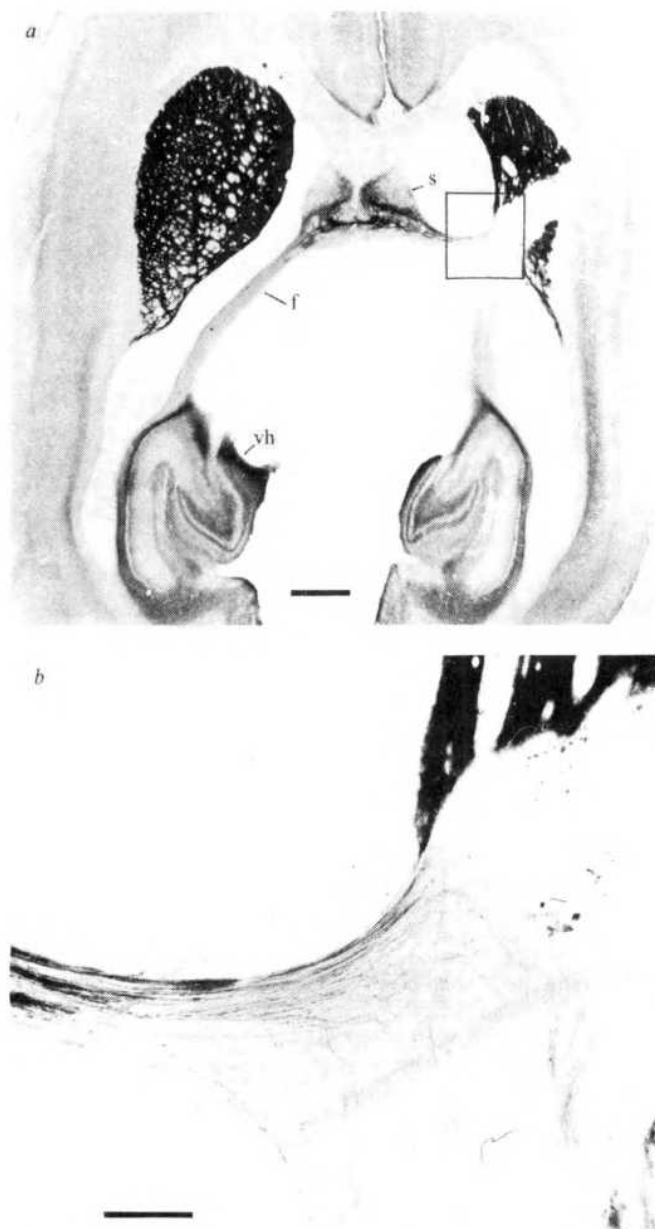


Fig. 2 *a*, Photomicrograph of AChE-stained, horizontal sections from rat brain 32 d after cotton swab-NaCl treatment of the fimbria. Section stained for AChE after the method of Matthews *et al.*¹³. The left side shows the normal course of fimbrial fibres travelling uninterrupted from the septum (*s*) to the ventral hippocampal formation (*vh*). The intact caudate nucleus is lateral to the fimbria. The right side shows the fimbria branching off towards the lesioned caudate nucleus. Although there is a disruption of fibres in the fimbria there is no depletion of AChE-staining in the ventral hippocampal formation. AChE-staining of the ventral hippocampal formation is almost completely eliminated by section of the fimbria⁸. In silver-stained material the branch is observed to contain many axons, of which only a small percentage stain positively for AChE. Calibration bar = 1 mm. *b*, Enlarged portion of boxed-in area of Fig. 2*a*, demonstrating AChE-positively staining fibres. Some of these fibres are shown to pass from the fimbria to contact the lesioned caudate in two separate regions, whereas other fibres continue posteriorly to the ventral hippocampal formation. Scale bar, 200 μ m.

fibres the extreme damage is a hindrance to growth. Second, the proximity of a lesioned caudate nucleus was apparently a condition for successful fibre outgrowth. In animals where most of the head of the caudate had been removed (the regions closest to the perturbed fibres of the fimbria) little, if any, growth of fimbrial fibres was observed. Other regions of close proximity to the fimbria, such as the thalamus, hypothalamus

and corpus callosum, were never targets for the outgrowing fibres.

We cannot specifically identify the nature of the perturbation that will initiate fibre growth. However, we feel that the cotton fibres from the swab, the mechanical disturbances of exposing the fimbria or the freezing of fimbrial fibres all act to irritate the fimbria and that this irritation induces nerve growth. The partially denervated cholinergic cells of the caudate nucleus may produce substances which attract and stimulate the growth of cholinergic fibres of the fimbria that have been prompted to grow. The role of factors such as fibre perturbations and the attractive influence of denervated tissues in stimulating and sustaining axonal growth is very similar to those factors hypothesised by Cajal, Nageotte and Marinesco to be critical for growth of axons in the periphery and spinal cord of the mammalian nervous system⁹. Seiger and Olson have shown that locus coeruleus neurones that have matured in the eye can reinitiate extensive fibre growth in response to contact by a graft of denervated iris tissue, but not following contact with a host iris that still has innervation¹⁰. In these studies denervation was seen to be the important factor in stimulating fibre outgrowth from the *in oculo*, mature locus coeruleus neurones. Similarly, caudate denervation may act to stimulate fibre outgrowth from the fimbria.

Our finding that disturbed but non-lesioned nerves in brain can be induced to grow is very similar to results obtained by Cajal in the young cat after spinal cord damage⁹. In fact, the presence of axonal varicosities, as we observed in our AChE-stained fibres, was noted by Cajal to occur in regrowing spinal nerve fibres. Speidel reported collateral growth in normal peripheral nerves of the tadpole¹¹. These nerve fibres sometimes formed entirely new pathways, induced by the denervation of a nearby region. Speidel also noted that this type of axonal growth seemed to be characteristic of unmyelinated axons. In this respect, Svendgaard *et al.*⁴ have suggested that the unmyelinated fibres of the fimbria have an ability to regenerate that may be shared by all unmyelinated axons in the central nervous system. They report a regenerative capacity of these nerves, although disturbance-induced growth (and not axotomy-induced growth) of the cholinergic fimbrial fibres could also account for their observations. Instances of neuronal remodelling that occur following regeneration, sprouting and growth evoked by mechanical perturbation as described here, may all rely on similar growth-inducing factors.

Our findings suggest that the consequence of denervation can extend beyond the immediate vicinity of damage and apparently stimulate the growth of axonal branches over a

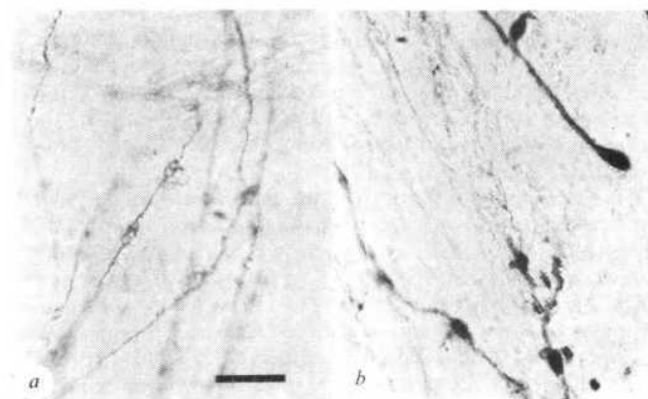


Fig. 3 *a*, Photomicrograph of AChE-positive axonal varicosities in fimbria branch 32 d after cotton swab-NaCl treatment of the fimbria. In cresyl-violet stained material these varicosities did not exhibit any staining. *b*, Photomicrograph of AChE-positive axonal varicosities 11 d after cotton swab treatment of the fimbria. Scale bar, 50 μ m.

distance a few millimetres from the denervated zone. This growth can apparently be initiated by several seemingly nonspecific mechanical disturbances of these nerves. Axonal growth from the fimbria may provide an opportunity to study the factors that effect initiation, stimulation and guidance of growing nerve fibres in the mature mammalian brain.

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Positive inotropism of vanadate in cat papillary muscle

MANY samples of 'Sigma grade' ATP from Sigma Chemical Co. reportedly contained an impurity which induces anomalous kinetics of (Na, K)-ATPase activity¹⁻³. This impurity has recently been identified as vanadate⁴, and shown to inhibit (Na, K)-ATPase from kidney⁴ and red blood cells⁵. These findings have received considerable interest because of a possible physiological role of vanadate as an endogenous regulator of (Na, K)-ATPase activity³⁻⁵. We now report that vanadate is capable of producing positive inotropic effects in electrically driven papillary muscles isolated from cats.

Cats (0.5-1.6 kg) were anaesthetized with sodium pentobarbital (30 mg per kg i.p.) and papillary muscles (mean cross-sectional area $0.62 \pm 0.05 \text{ mm}^2$, $n = 17$) were dissected from the right ventricles. The preparations were attached to a platinum stimulating electrode and mounted individually in glass tissue chambers for recording isometric contractions as described previously⁶. The bathing solution (5 ml) containing (mM) NaCl 136.9, KCl 5.4, CaCl₂ 1.8, MgCl₂ 1.05, NaH₂PO₄ 0.42, NaHCO₃ 11.9, glucose 5.5 was equilibrated with 95% O₂ + 5% CO₂ and maintained at 35°C. The preparations were driven electrically at a frequency of 0.2 Hz (duration 5 ms, intensity about 10% above threshold). Drugs used were ammonium vanadate anhydrous (NH₄VO₃), sodium vanadate anhydrous (NaVO₃; both from Merck, Darmstadt) and ($\pm$)propranolol HCl (ICI). The compounds were freshly dissolved in bathing medium and injected into the tissue chamber in volumes of 300 μ l or less. NH₄VO₃ and NaVO₃ did not contain any measurable calcium and did not change the pH (7.4) of the bathing solution.

The effects of NH₄VO₃ on force of contraction are illustrated by the representative experiment shown in Fig. 1. NH₄VO₃ (20-500 μ M) was added cumulatively, and the time of exposure

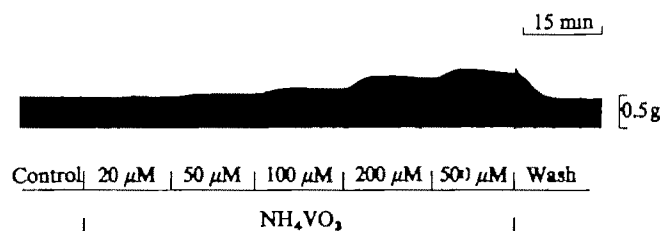


Fig. 1 Effect of NH₄VO₃ on isometric force of contraction of a cat isolated papillary muscle driven electrically at a frequency of 0.2 Hz. NH₄VO₃ was applied cumulatively at concentrations of 20-500 μ M as indicated. The time of exposure to each concentration of NH₄VO₃ was about 15 min. After exposure to 500 μ M NH₄VO₃, the preparation was washed three times with drug-free bathing solution. Temperature 35°C. Extracellular Ca²⁺ concentration 1.8 mM. Preparation diameter 0.7 mm. Experiment 13 06 784. Similar results were obtained in each of nine other preparations.

to each concentration was about 15 min. NH₄VO₃ produced a concentration-dependent increase in force of contraction. The effect became clearly visible at 50 μ M and was maximal at 500 μ M. These concentrations correspond to those of Na₂VO₄ required to inhibit rubidium uptake in intact red cells by 50 to 80%⁵. The typical time course of action is also well shown. An increase in force of contraction was detectable after about 1 min, and a peak effect was attained after 6-3 min. The effect of NH₄VO₃ was readily reversible within less than 15 min on washing with drug-free bathing solution. Higher concentrations of NH₄VO₃ (1 mM) produced arrhythmias as a sign of toxicity.

The mechanism of the positive inotropic effect of NH₄VO₃ remains to be elucidated. Similar results as with NH₄VO₃ were obtained with NaVO₃ and, moreover, the effect of NH₄VO₃ was also observed in the presence of 1 μ M of the β -adrenoceptor blocking agent propranolol (data not shown). This indicates that the positive inotropic action of NH₄VO₃ was neither due to an effect of the NH₄⁺ ion nor to a stimulation of β -adrenoceptors. Experiments are in progress to determine whether the positive inotropic effect of vanadate is accompanied by an inhibition of myocardial (Na, K)-ATPase, and, if so, whether both effects occur at similar concentrations of vanadate.

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Table 1 Allocation of *Est-5* variants to charge classes

Charge class	Frequency*	<i>Est-5</i> variant
+2	0.012	0.85
+1	0.094	0.89, 0.89 ^a , 0.94, 1.00 ^c
0	0.457	0.97M, 1.00, 1.00 ^b , 1.00 ^d , 1.00 ^e , 1.00 ^f , 1.02, 1.03
-1	0.220	1.06, 1.06 ^g , 1.06 ^c , 1.06 ^h , 1.09
-2	0.027	1.16
Others		0.98, 1.04, 1.12

*Frequency values given are pooled frequencies of the individual variants as given by Prakash¹⁶.

non-standard electrophoretic conditions^{11,13}, only two, 1.00^c and 1.00^e, were resolved from the other variants in their standard classes by isoelectric focusing. One of these, 1.00^c, which is separable from other 1.00 variants by its slower mobility on 14% starch gel at pH 6.8 (ref. 11), occupied the same position as the 0.94 variant on isoelectric focusing.

Esterase-5 is a dimeric enzyme and the positions of some heterodimers are depicted in Fig. 1. Heterodimers are formed readily during electrophoresis of mixtures of homodimers. The 0.97 M variant behaves as a monomer in all conditions, and does not form stable heterodimers with the subunits of other variants.

The position of most variants after isoelectric focusing can be interpreted in terms of the charge-state model (Table 1). There are five main charge classes characterised by an essentially equal spacing. Three variants (0.98, 1.04, 1.12) exhibit very anomalous behaviour on isoelectric focusing and also do not fit the proposed charge classes; they are discussed below. The positions of the 1.16/1.00 and the 1.06/0.94 heterodimers show that these four variants are separated by equal charge changes. We propose that this is the result of substitutions which are approximately one unit charge jump apart. Small variations in charge are observed between the variants within single charge classes, but these variations in charge are small compared to the postulated unit charge jump. The apparent charge identity after isoelectric focusing of variants, which can be distinguished by standard conditions of electrophoresis (such as 1.06 and 1.09), suggests that differences due to apparent conformational changes are being detected by standard electrophoresis. Shifts in relative mobility could also represent small changes in net charge between variants being differentially expressed at the different pH conditions of electrophoresis.

The pooled frequencies of the variants within each major charge class fit a charge class configuration expected for neutral alleles with the central classes being most frequent (Table 1).

As pointed out above, the 1.00^c variant is not resolved from the 0.94 variant using isoelectric focusing. It is postulated that a substitution, resulting in an extra histidine residue, separates this variant from the other 1.00 variants. At the isoelectric point for *Est-5* (approximately pH 5.0), the 1.00^c variant would then jump charge classes; the size of this jump is consistent with our postulated unit charge change.

Considering their relative positions on isoelectric focusing, three variants (0.98, 1.04 and 1.12) display much faster mobilities in standard electrophoretic conditions than would be expected from their charges. These three variants lie approximately halfway between the postulated charge classes. They are evenly spaced and show the same charge jump increment as the major charge classes. These results suggest that they differ from each other by single charge changes, and may possess common features which separate them, as a group, from the variants which form the major charge classes.

Johnson¹⁴ has examined the charge-state model for *Est-5* indirectly by the use of gel-sieving. He claims that the combination of the free electrophoretic mobilities and retardation effects coincidentally result in apparent unit increments in mobility at pH 8.9. He concluded that the seven variants which he examined fit a charge class configuration; however, he did not study a

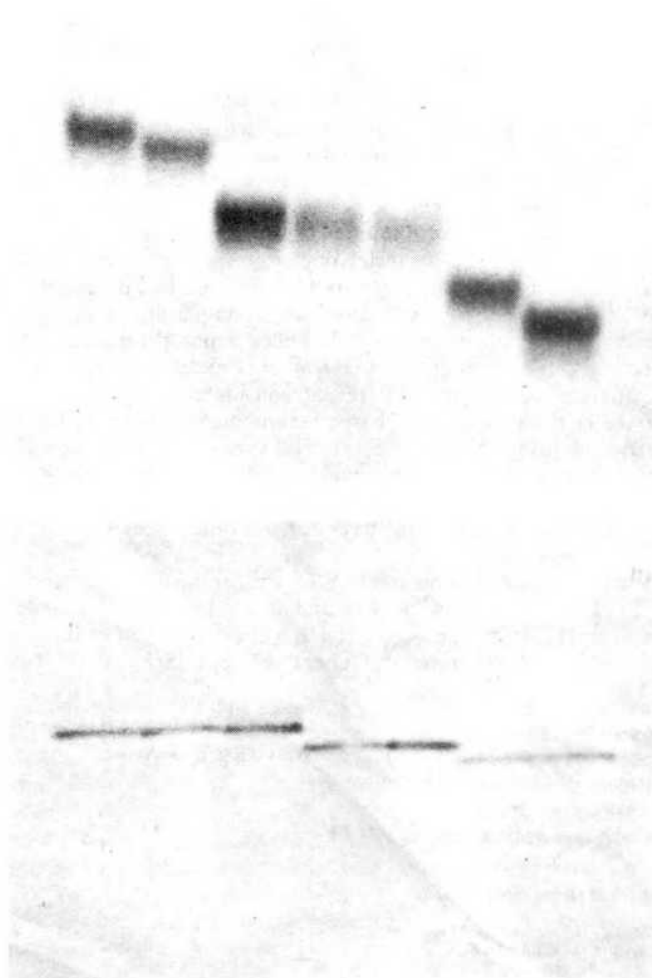


Fig. 2 Top: electrophoresis of (0.89, 0.94, 1.00^c, 1.00, 1.02, 1.06 and 1.09) *Est-5* variants in 5% acrylamide gel pH 8.9 (ref. 9). Bottom: isoelectric focusing of (0.89, 0.94, 1.00^c, 1.00, 1.02, 1.06 and 1.09) *Est-5* variants. Isoelectric focusing was based on the method of Vesterberg¹⁷, using 5% gels, 18 × 18 cm, polymerised by ammonium persulphate. Gels contained 1.6% w/v pH 4–6 and 0.4% w/v pH 3–10 ampholytes. Electrodes of Pt wire between Whatman 3MM paper were 16 cm apart; anodic and cathodic solutions were 0.2% v/v sulphuric acid and 0.4% v/v ethanolamine respectively. Samples, 10–15 μl, were loaded on 5 × 5 mm Whatman 3MM paper placed 2 cm from the cathode. Electrophoresis was at 4 °C for 4 h.; the voltage was gradually raised to 500 V over 1 h and maintained at this value. pH measurements were made at 20 °C on ampholytes eluted from transverse sections of gel. Staining for esterase activity was by the standard method¹³.

number of fairly common variants of intermediate mobility. Johnson claimed to find extensive genetic heterogeneity within allelic classes. He also found allelic heterogeneity in certain strains (some believed isogenic), yet no heterozygotes were found in these strains, which seems unlikely. More recently, Cobbs and Prakash¹⁵ examined the fit of *Est-5* to a charge state configuration using gel-sieving, and by chemically modifying the charge on several reference proteins. Using the average change in mobility of each reference protein per unit change in charge, they estimated an overall average mobility change per unit charge of 0.046 ± 0.029 . Chemical modification of charge on *Est-5* was not carried out. They assumed that the overall average was applicable to *Est-5*. As the magnitude of each charge change will vary with protein size and the proportion and balance of its charged residues, we consider it fortuitous that their estimate derived from diverse proteins was so close to our postulated unit charge change. The results of the studies by

Johnson¹⁴, and Cobbs and Prakash¹⁵, both using indirect methods of charge estimation, are incompatible with each other, and with the results of this study.

Our study of the differences in charge balance between *Est-5* variants shows clearly that *Est-5* can be fitted to the charge-state model. Despite the large numbers of *Est-5* variants segregating in natural populations, the total variability collapses into two offset charge class configurations, of five and three classes. There does not seem to be a continuum of charge change as claimed by Johnson⁶, but rather a clustering of charge variants around unit charge increments. Electrophoretic variation within charge classes and between the two major charge configurations may be the result of conformational differences exposed on electrophoresis. Our study indicates that conventional electrophoresis, in different conditions, will reveal considerably more genic variability than the simple charge heterogeneity shown by isoelectric focusing. Nevertheless, allelic variation at a complex locus such as *Est-5*, can be more clearly understood by the application of isoelectric focusing. Experiments are in progress to determine the structural basis for the differences between various *Est-5* variants.

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Bile salt sensitivity of *Escherichia coli* χ 1776

Escherichia coli χ 1776¹, a 'biologically contained' (EK2) host specifically constructed for genetic manipulation (ref. 1 and R. Curtiss, III, personal communication) contains many mutations which decrease its survival characteristics compared with wild-type *E. coli* and which also prevent its colonisation of the intestinal tract of mammals. The bile salt sensitivity of χ 1776 is one of the parameters which preclude its survival in the intestine. This sensitivity is related to modifications of the cell envelope structure but, more specifically, to mutations at the *oms-1* or *rfb-2* loci. Curtiss (personal communication) has reported that the reversion frequency of χ 1776 to bile salt resistance is approx. 10^{-7} to 10^{-9} . However, the cell surface structures of bacteria are subject to phenotypic variation and under control of a number of genes. In this paper we describe experiments which indicate that the bile salt sensitivity of χ 1776 is phenotypically variable.

Batch cultures of χ 1776 in L broth were monitored for bile salt resistance at 16 and 40 h after the initiation of growth. The number of bile salt-resistant organisms detected was constant,

Table 1 Bile salt resistance of χ 1776 in batch cultures

Inoculum size per 100 ml culture	Time after inoculation (h)	Proportion of resistant organisms	Culture pH
5×10^7 organisms	16	1 in 10^4 - 10^5	6.5
5×10^7 organisms	40	1 in 10 - 10^2	8.0
10^9 organisms	16	1 in 10 - 10^2	8.0

χ 1776 was grown in L broth (1% Bacto-Tryptone, 0.5% yeast extract, 0.5% NaCl, 0.1% glucose, supplemented with 0.01% diaminopimelic acid, 0.004% L-threonine, 0.001% L-methionine, 0.0001% biotin and 0.004% thymidine). The initial pH of the culture medium was adjusted to 7.0 by addition of 1 M NaOH. The bile salt sensitivity of different cultures of χ 1776 was monitored by plating out duplicate samples (0.2 ml) on L-agar and MacConkey agar supplemented with the specific growth requirements of the strain as given above. Essentially identical results were obtained with supplemented MacConkey agar plates containing 0.15% (Difco and Oxoid no. 3 formulations) and 0.5% (Oxoid no. 5 formulation) bile salts.

within experimental error, over a number of experiments but was dependent on the time of incubation at 37°C, the initial inoculum size and the final pH of the culture supernatant (Table 1).

The variation in the culture pH indicated that the organism initially utilised the glucose present (0.1%) to produce acid. Depletion of glucose resulted in the utilisation of amino acids as carbon sources and in an increase in culture pH due to the secretion of ammonia. Differences in the numbers of bile salt-resistant organisms found here in primary cultures (low inoculum sizes and short incubation times) and those found by Curtiss (1 in 10^7 - 10^9) may be due to differences in the batches of medium used. Nevertheless, these results indicate that the bile salt sensitivity of χ 1776 is dependent on culture conditions and that the surface properties of this organism might be subject to phenotypic change. The effect of growth rate on the bile salt sensitivity of χ 1776 was therefore investigated in an attempt to gain further information. This was of additional interest as the mean generation time of *E. coli* in the mammalian intestine is considerably longer (12-24 h) than that observed using conventional laboratory techniques (0.5-1.5 h)^{3,4}.

A chemostat culture was set up so that χ 1776 could be grown at a very low rate (Table 2). When steady-state conditions were reached (5 d) the culture was shown to be glucose limited (that is no glucose was present in the culture supernatant and addition of glucose to the chemostat produced a corresponding increase in cell density); the culture pH was uncontrolled and was 8.2. One in two of the organisms present were bile salt-resistant in these conditions. The dilution rate was then increased and at each steady state the number of bile salt-resistant organisms and the pH of the culture was measured. The proportion of bile salt-resistant organisms present in these different conditions remained approximately constant even though the culture pH, as expected, fell as the dilution rate increased (Table 2). This indicated that a bile salt-resistant variant had been selected.

In an additional experiment a chemostat culture of χ 1776 was grown to steady-state conditions at a dilution rate of 0.025 h^{-1} , the culture pH being maintained at 6.0 by addition of 2 M HCl. The bile salt resistance of this culture remained at its starting level of 1 in 10^4 . The culture pH was then adjusted to 8.0 by addition of 2 M NaOH and the bile salt resistance monitored as a function of time. In less than one mean generation time, one in four of the organisms present had become bile salt resistant. This rate of change in the population in the chemostat indicates that the bile salt sensitivity of χ 1776 is subject to phenotypic variation. After 10 d (during which the proportion of resistant organisms remained at one in four) the culture was returned to pH 6.0 by addition of 2 M HCl and a new steady state was established. Approximately one in ten organisms in the culture remained bile salt resistant, indicating that selection of a resistant variant had again occurred and that this variant was rela-

tively stable. The growth of a single colony isolate of the bile salt-resistant variant in batch culture confirmed this stability. The variant grew well at pH 8.0, 7.1 and 6.3 in the presence of at least 0.25% bile salts and in the presence of nalidixic acid $50 \mu\text{g ml}^{-1}$. Furthermore, growth at pH 8.0 in the absence of bile salts produced a culture in which less than one in 10^4 organisms were bile salt sensitive. This increased to approximately one in 2×10^2 when the variant was grown at pH 6.3.

Throughout these experiments, lasting a number of mean generation times, no variation in the absolute requirements for diaminopimelic acid and thymidine were observed. Indeed, the cultures remained phenotypically $\chi 1776$ except for the variations detected in bile salt sensitivity. There had therefore been no contamination with other *E. coli* strains.

Table 2 Bile salt resistance of $\chi 1776$ in chemostat cultures

Dilution rate	Mean generation time (h)	pH of culture	Proportion of resistant organisms
Initial batch culture	1 (in log growth)	7.0 → 6.5	1 in 10^4
0.025	26	8.5	1 in 2
0.05	13	8.2	1 in 5
0.1	7	7.7	1 in 5
0.3	2	7.5	1 in 10
0.6	1.1	6.4	1 in 10
Wash-out point			

A Porton-type chemostat (500 ml L broth containing 0.1% glucose and growth supplements) was inoculated with $\chi 1776$ and this was grown at 37°C in batch culture conditions; after 16 h the chemostat was switched to the continuous culture mode with the dilution rate set at 0.025 h^{-1} (mean generation time 26 h). The pH was monitored but not controlled. The dilution rate was then increased and the number of bile salt-resistant organisms was determined at each new steady state. The culture density remained at approximately 5×10^9 – 10^{10} organisms ml^{-1} until wash-out.

These experiments do not necessarily preclude the use of $\chi 1776$ as a host in genetic manipulation. They do, however, show that, if care is not taken, selection for variants of this organism may occur. These variants, with slightly better growth characteristics, can quickly outgrow 'authentic' $\chi 1776$ produced by serial passage in batch cultures. In addition, bile salt-resistant variants of $\chi 1776$ have slightly better survival characteristics in animal tests and are better recipients for some conjugative plasmids (R. Curtiss, III, personal communication). These results indicate that any form of biological containment that is dependent on the surface properties of *E. coli* may be suspect.

Finally, and more importantly, these experiments demonstrate the need to monitor the safety features of any 'biologically contained' organism before, and during any genetic manipulation experiment.

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Expression of λ red genes restores *recB*-dependent protein X induction in *E. coli*

DNA DAMAGE and/or inhibition of DNA synthesis elicit a number of responses in *Escherichia coli* known as SOS functions¹, including the production of large quantities of a protein provisionally called protein X (ref. 2). Induction of protein X, like the other SOS functions, requires functional *recA* and *lexA* genes^{3,4}. Gudas and Pardee⁴ proposed a model to suggest roles for *recA* and *lexA* in the regulation of protein X production. Several research groups have identified protein X as the *recA* gene product^{5–8} which prompted them to formulate updated models, to account for the apparent autoregulation of the *recA* gene^{6–8}. These models propose that the *lexA* gene codes for a repressor which inhibits the transcription of the *recA* gene. Following treatment, such as exposure to ultraviolet irradiation or nalidixic acid, a small inducer molecule is produced that causes the *recA* gene product, present in low levels in untreated cells, to inactivate the *lexA* repressor. This allows transcription of the *recA* gene that results in the production of large amounts of the *recA* gene product, protein X. Gudas and Pardee⁴ suggested that the inducer molecule may be a DNA degradation product resulting from the action of the *recBC* gene products, exonuclease V. In support of this idea they showed that protein X is not induced by nalidixic acid in *recB*[–] mutants deficient in exonuclease V. On the other hand, Little and Hanawalt⁹ showed that intracellular degradation of unmodified phage DNA by restriction is not sufficient to induce protein X, and they have challenged the role of DNA degradation in the induction process. In this report we show that phage λ genes controlling λ exonuclease can restore the missing function for the induction of protein X in *recB*[–] mutants. This is further evidence that DNA degradation is required to derepress the *recA* gene.

As the exonuclease V protein has an ATPase activity in addition to both endo- and exonuclease activities¹⁰, the missing function for the induction of protein X in nalidixic acid-treated *recB*[–] mutants could be some function other than that of degrading DNA. We have therefore tried to determine whether λ exonuclease, an exclusively exonucleolytic enzyme¹¹, could circumvent the *recB* mutation in the induction of protein X. To do this we have determined whether protein X was induced in *recB*[–] lysogens that were transiently induced to produce λ exonuclease before treatment with nalidixic acid. In similar experiments others have shown that transient prophage induction enhanced *recB*-dependent cell survival after ultraviolet¹² or X-ray irradiation¹³.

Figure 1 shows the effect of transient heat induction of λ cI857 prophage on protein X induction in nalidixic acid-treated *recB*[–] lysogens. Lanes 8–12 show that the amount of protein X increased with increasing times of incubation of 42°C . In control experiments shown in lanes 3 and 4, neither a 5-min incubation at 42°C nor nalidixic acid treatment alone was sufficient to induce protein X. To investigate the role of λ exonuclease in this induction, the above experiments were repeated using *recB*[–] lysogens carrying prophage with mutations in genes controlling λ exonuclease activity. The combined treatment of heat induction and exposure to nalidixic acid was not sufficient to induce protein X when the prophage carried the mutations *redX314*, (lane 6) or *redB113* (lane 7). *redX314* and *redB113* are point mutations in the genes which code for λ exonuclease and β protein, respectively^{14,15}. λ exonuclease acts primarily at 5'-phosphoryl termini on double-stranded DNA molecules, and the only acid-soluble products are deoxyribonucleoside 5'-monophosphates¹¹. The *redX314* mutation decreases the activity of λ exonuclease to less than 2% of normal activity¹⁴. Although the *redB113* mutation does not affect the λ exonuclease enzyme, it does alter the antigenic properties of the

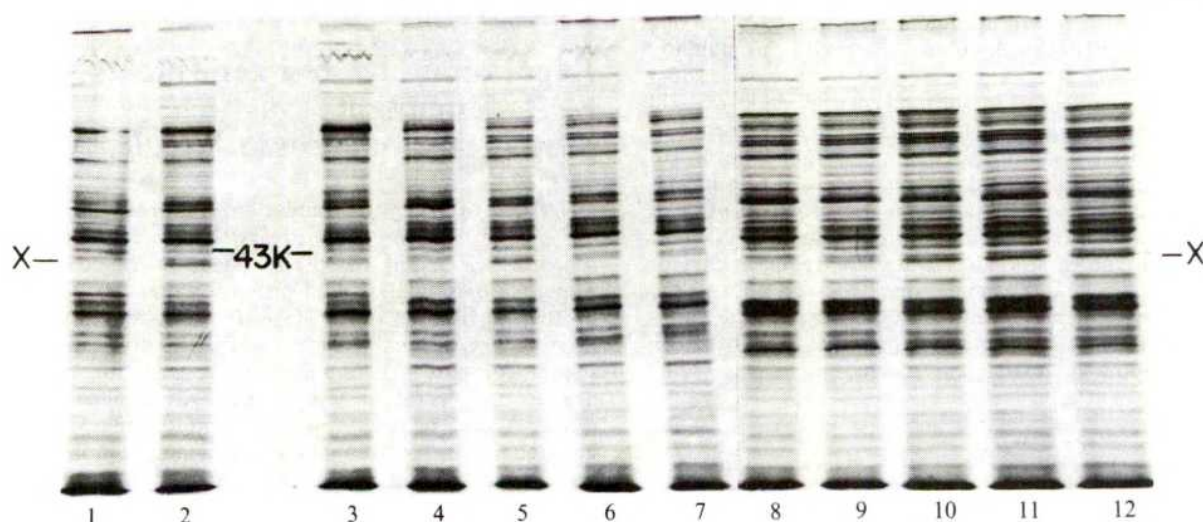


Fig. 1 Strain AB2470 *recB21* (ref. 21) was lysogenized with λ CI857, λ CI857 *redX314* or λ CI857 *redB113*. The λ CI857 mutant produces a temperature-sensitive repressor. The *redX314* and *redB113* mutations are described in the text. Lysogens were grown at 30 °C to an $A_{590} = 0.2$ in glucose-M9 media containing required amino acids and thiamine; 1-ml samples were incubated at 42 °C for various times before addition of nalidixic acid, 50 $\mu\text{g ml}^{-1}$ (Sigma). At 30 min after addition of the drug, ^{14}C -leucine (Schwarz-Mann) was added (to 5 $\mu\text{Ci ml}^{-1}$) and incubation was continued for 60 min at 30 °C. The cells were collected by centrifugation and prepared for SDS-polyacrylamide gel electrophoresis as described by Little and Hanawalt⁹. This figure is a composite picture of autoradiographs of two slab gels. Equal amounts of radioactivity were added to each slot in each gel. All lanes except 6 and 7 show proteins synthesised in AB2470 carrying λ CI857 prophage, unless otherwise noted, that were treated as described below. Untreated (lane 1); irradiated with 30 J m^{-2} UV (lane 2); incubated at 42 °C for 5 min (lane 3); exposed to nalidixic acid, 50 $\mu\text{g ml}^{-1}$ (lane 4); incubated at 42 °C for 5 min followed by exposure to nalidixic acid (lane 5); AB2470 (λ CI857 *redX314*) treated as in lane 5 (lane 6); AB2470 (λ CI857 *redB113*) treated as in lane 5 (lane 7); incubated at 42 °C for 1 min (lane 8), 2 min (lane 9), 3 min (lane 10), 4 min (lane 11) and 5 min (lane 12) followed by exposure to nalidixic acid.

β protein¹⁵. The only known activity of the β protein is to enhance the affinity of λ exonuclease for DNA *in vitro*¹⁶. This evidence indicates that the activity of λ exonuclease is responsible for the induction of protein X in nalidixic acid-treated *recB*⁻ lysogens. We interpret this to mean that DNA degradation is necessary for nalidixic acid-mediated induction of protein X.

As SOS functions, such as cell filamentation, error-prone DNA repair, and λ prophage induction, are dependent on the *recA*⁺ *lexA*⁺ genotype, the derepression of the *recA* gene seems to be necessary for their expression. For this reason, SOS functions are elicited by treatments that result in DNA degradation. How does the *recA* protein activate SOS functions? Roberts and Roberts¹⁷ have shown that λ prophage induction is due to a *recA*-dependent proteolytic cleavage of the λ repressor. They have recently found that the *recA* gene product, protein X, cleaves the λ repressor *in vitro* (J. W. Roberts, personal communication). Witkin¹⁸ proposed that all of the SOS functions are derepressed by a common mechanism, is by proteolytic cleavage of the repressor(s) controlling the SOS operon(s). DNA degradation, an initial consequence of DNA damage or of a stalled replication fork, may provide a primary danger signal which is amplified through the action of the *recA* protein resulting in the expression of a set of functions designed to enhance cell survival.

Although the above evidence clearly indicates that *recB*-dependent DNA degradation is required for nalidixic acid induction of protein X, this dependence may not apply to induction by other agents. For example, lane 2 of Fig. 1, indicates that ultraviolet induction of protein X is independent of the *recB* mutation, confirming the results of Little and Hanawalt⁹. Perhaps excision of ultraviolet photoproducts or post-replication repair results in sufficient DNA degradation, not requiring the *recBC* exonuclease, to trigger the derepression of the *recA* gene. In support of this suggestion, Braun¹⁹ has shown that ultraviolet induction of λ prophage does not occur if both excision and replication are eliminated genetically. Thus, we propose that more than one pathway of DNA degradation may lead to the induction of SOS functions.

What is the identity of the inducer? An attractive idea is that a modified DNA degradation product serves as a specific signal to communicate to the cell that its genetic material is in jeopardy, and that the SOS functions are needed. This would explain the results of Little and Hanawalt⁹ that degradation of unmodified phage DNA is not sufficient to cause induction. As modification is a post-replication process²⁰, a modified DNA degradation product could unequivocally be distinguished from DNA precursors normally present in the cell. Moreover, such a role for modification in the induction of SOS functions would emphasise the importance of pathways for the preservation of genetic material and would provide considerable insight into the cellular function of DNA modification.

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Haem exposure as the determinate of oxidation-reduction potential of haem proteins

KASSNER¹ has proposed that the apolarity of the haem environment primarily determines the oxidation-reduction potential, E'_0 pH 7.0, of a haem protein. Since high resolution crystallographic models are now available for six haem proteins with E'_0 values of between 20 and 320 mV, I have tested the Kassner proposal by comparing the kinds of atoms contacting the haem in each of these proteins. The results demonstrate that the apolarity of the total haem environment for these proteins is virtually identical but that the E'_0 value is inversely dependent on the exposure of the haem to an aqueous solvent.

Surface area calculations were made using a DEC PDP 11/45 computer and protein coordinates supplied by the Brookhaven Protein Data Bank. An unpublished computer program developed by Arnone and Briley (similar to that of Shrake and Rupley²) to both generate and display protein surfaces accessible to water solvent molecules was used. Each atom in a protein is represented by a sphere centred about its atomic coordinates with radius the sum of its Van der Waals radius and 1.4 Å, the radius of a water molecule. A three-dimensional grid of equally spaced points is constructed large enough to envelop the entire hydrated protein molecule. Grid points which lie within the space defined by spheres centred at each atom are termed interior points. The six nearest neighbours of each interior point are then examined. Those interior points which are not bounded on all sides by other interior points are identified as surface points. Calibration experiments have shown that the accessible surface of a sphere representing a typical hydrated protein atom is sampled by about 300 points using a 0.5 Å grid and results in a random calculation error of less than 5%. All surface points which lie on the surface of only one atom are assigned to that atom, while surface points shared by more than one atom are uniquely assigned to the atom having the nearest centre. The total number of surface points associated with each atom is converted to Å² of surface area using the factor 3.41 surface points Å⁻² obtained from a calibration curve. Carbon and sulphur atoms are considered as apolar atoms while all other haem protein atoms are considered as polar atoms.

All surface area calculations reported here were made using a 0.5 Å grid. The surface area of each haem and each apoprotein

was obtained by elimination of all coordinates associated with the apoprotein and the haem atoms, respectively, prior to calculation. The surface area of each haem crevice was calculated by insertion of the haem surface into the protein surface, removal of all points common to this composite surface and to the haem protein and assignment of the points remaining to individual atoms as above.

The total accessible surface area of the crystallographic model for each of the six haem proteins considered is listed in Table 1, entry 1. These surface areas are related to molecular weight by the expression, surface area = $11.0 M^{2/3}$, in excellent agreement with the equation developed by Teller³ using the surface areas calculated for proteins by the procedure of Lee and Richards.⁴ Approximately half ($53 \pm 3\%$) of the accessible surface area of these haem proteins is contributed by apolar atoms, entry 2, a percentage within the range calculated for five proteins by Lee and Richards³ and by Shrake and Rupley². These favourable comparisons indicate that our procedures for calculations of surface area and assignment of area to individual atoms are consistent with results obtained by others.

Removal of the coordinates for the apoprotein atoms from each of the haem proteins and subsequent surface area calculation, entry 4, indicates that a haem constructed from protoporphyrin IX has an accessible surface area of 805 ± 15 Å². As shown in Table 1, entry 3, between 77 and 96% of haem surface is buried in the haem proteins considered. Inspection of the haem atoms which are accessible in the haem proteins indicates (1) that neither the iron atom nor any of the pyrrole or methine bridge atoms are accessible, (2) that the atoms comprising some of the methyl, vinyl and propionate side chains are accessible, and, (3) that the haem is orientated in these haem proteins in one of two distinctive modes exposing different side chains as illustrated in Fig. 1. Haems which are not covalently attached to the polypeptide chains as in haemoglobin, myoglobin and cytochrome *b₅*, are orientated such that the side chain pyrrole rings 3 and 4 are accessible to the solvent, presumably to place the propionate carboxylates in a more polar environment. By contrast, haems which are covalently attached to polypeptides by addition of cysteinyl sulphhydryl groups across vinyl side chains, as in the cytochromes *c*, appear to be rotated by about 90° exposing the vinyl group of pyrrole 2 and the methyl groups of pyrroles 2 and 3. This orientation which buries the two propionates, would be expected to require complementary pairing of the buried carboxylate oxygen atoms by polar atoms in the haem crevice.

Table 1 Accessible surface area calculations of haem proteins

Entry	Parameter	Cyto <i>c</i> ₂	Cyto <i>c</i>	Cyto <i>c</i> ₅₅₀	Hb _α	Hb _β	Myo	Cyto <i>b</i> ₅
Haem protein								
1	Total accessible surface	5,473	5,307	6,613	6,770	7,152	7,120	4,858
2	Accessible apolar surface	3,019	2,860	3,414	3,849	3,760	4,000	2,352
3	Accessible haem surface	49	32	38	116	163	144	176
Haem								
4	Total accessible surface	796	819	832	800	805	799	783
Haem crevice of apoprotein								
5	Total accessible surface	740	782	784	677	632	645	593
6	Accessible apolar surface	504	550	623	534	516	509	474
7	Accessible aromatic apolar surface	200	140	158	150	146	137	118
Tabulations								
8	% Haem exposed in haem protein	6	4	5	14	20	18	23
9	% Haem crevice apolar	68	70	79	79	82	79	80
10	% Haem crevice aromatic	40	25	25	28	28	27	25
11	% Haem environment apolar	63	67	75	66	64	64	61
Reduction potential								
12	E'_0 , pH 7 (mV)	320 ⁶	260 ⁷	250 ⁸	113 ⁹	53 ⁹	47 ^{9,10}	20 ¹¹

The protein column headings reading from left to right refer to cytochrome *c*₂ from *Rhodospirillum rubrum*, ferricytochrome *c* from tuna heart, cytochrome *c*₅₅₀ from *Paracoccus denitrificans*, the α₁ subunit of human deoxyhaemoglobin, the β₂ subunit of that protein, sperm whale metmyoglobin, and cytochrome *b*₅ from bovine liver. Surface areas are expressed in Å².

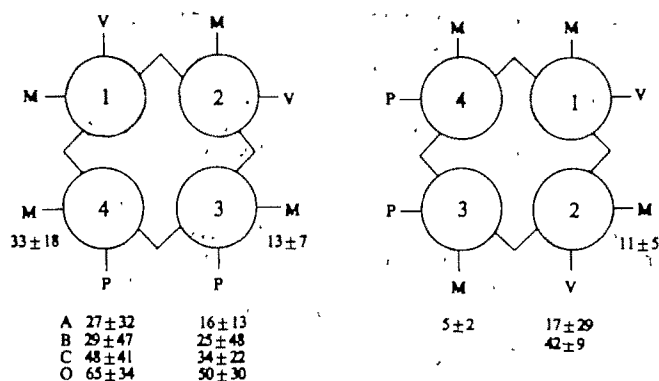
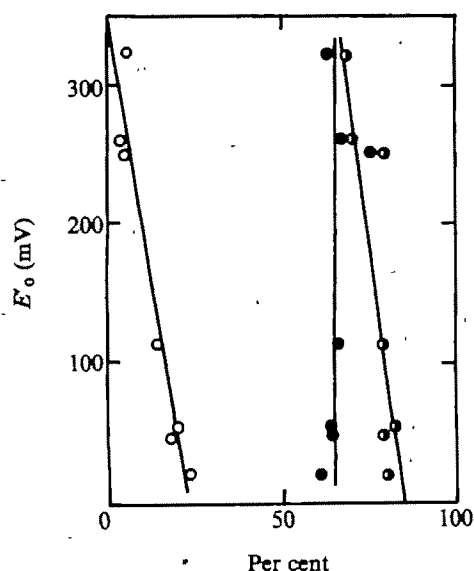


Fig. 1 Haem orientations in haem proteins. The haem present in haemoglobin, myoglobin and cytochrome *b*₅ is represented on the left and the haem in cytochrome *c* on the right. The pyrrole rings represented by circles numbered 1-4 and the pyrrole side chains represented by M, V and P for methyl, vinyl and propionate groups, respectively. Each haem is orientated such that its exposed side chains are at the bottom. The mean percentile exposure and standard deviation for the exposure of haem atoms in the haem proteins relative to their exposure in haem itself are listed below the atoms concerned. A, B, C and O refer to the exposure of the carbon atoms, A-C, and the oxygen atoms, O, of methyl, vinyl and propionate groups reading outwards from the pyrroles considered.

As shown in Table 1, at least 98% of the total accessible surface area for each haem, entry 4, can be accounted for by the sum of its accessible surface area in the haem protein, entry 3, and the accessible surface area of its crevice, entry 5. More than half, 61 ± 15%, of the crevice polar surface is contributed by polypeptide backbone oxygen and nitrogen atoms. By contrast, only 8 ± 3% of the crevice apolar surface is contributed by polypeptide backbone carbon atoms. No good correlation exists between the E'_0 value and the percentage contribution of the atoms of any amino acid or groups of amino acids, for example aromatic apolar atoms, entry 10, to either the polar or apolar crevice surface. The apolar side chain atoms of Phe, Ile, Leu and Val residues contribute at least 50% of the crevice apolar surface while those of Asp, Glu, Asn, Gln and Arg contribute less than 8% in keeping with the occurrence of these residues in protein interiors⁵.

Fig. 2 Dependence of reduction potential, E'_0 , on the per cent exposure of the haem (○), on the per cent apolarity of the haem crevice (●) and on the per cent apolarity of the haem environment (●). All values were obtained from Table 1. Each line represents a least squares analysis of the values shown.



The dependence of the reduction potential on the haem exposure, on the apolarity of the haem crevice and on the apolarity of the haem environment (Table 1, entries 8, 9, and 11) are compared in Fig. 2. The burial of the haem is accompanied by a nearly parallel decrease in the apolarity of the haem crevice. This parallelsim seems reasonable since burial of one or both haem propionates and/or provision of the second extrinsic haem ligand by the protein would require complementary polar atoms in the haem crevice as noted above. The net effect of these parallel changes is to maintain the apolarity of the haem environment constant while the reduction potential varies by 300 mV as shown in Fig. 2. This observation is clearly contrary to the Kassner proposal.

As shown in Fig. 2, the reduction potential is inversely related to the fraction of haem surface exposed according to the expression $E'_0 = -15 (\% \text{ exposure}) + 345 \text{ mV}$. This empirical relationship may reflect the accessibility of the haem to environmental O_2 or the proximity of the extrinsic ligands to the haem iron. As more of the protein envelops the haem, the ferrohaem would be more protected from oxidants and/or the haem iron-extrinsic ligand bond distance would be diminished. Both of these changes should increase the reduction potential of a haem protein¹².

The equation relating E'_0 and haem exposure merits additional comment. This equation predicts that a totally exposed haem would have an E'_0 value of -1,155 mV and that a totally buried haem a value of +345 mV. This range encompasses the E'_0 values reported^{13,14} for the majority of haem proteins except for cytochrome oxidase, cytochrome *f* and a few cytochromes *c*₅₅₃ and *c*₅₅₂, whose values extend to +400 mV. The equation is inappropriate for cytochrome oxidase since its haem moiety, haem A (ref. 15), has a larger surface area due to the presence of a farnesyl side chain. A very modest increase in the slope of the line shown in Fig. 2 would raise the maximum E'_0 value to +400 mV and thus accommodate the remaining measured values. Unfortunately, the E'_0 value for a monomeric haem in aqueous solution cannot be measured experimentally since haems avidly polymerise by base stacking and/or micelle formation. The E'_0 value for the monomeric haem octapeptide of cytochrome *c* has been reported¹⁶ to be -217 mV. The equation predicts that about 37% of the surface of this haem would be buried by the peptide atoms. While the structure of the haem octapeptide is unknown, a CPK model indicates that the predicted burial is feasible. Thus, the exposure of the haem moiety, rather than the apolarity of the haem environment or the ligands in the fifth and sixth haem coordination position seems to be the principal determinant for the magnitude of the E'_0 value.

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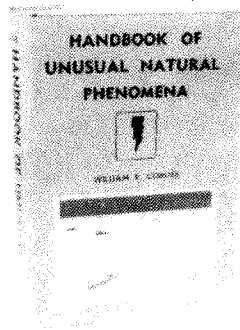
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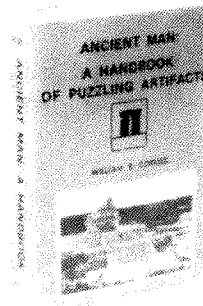
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reviews

Prehistoric standing stone sites

E. MacKie

Sun, Moon and Standing Stones. By J E Wood Pp 217. (Oxford University Press: Oxford, 1978) £6.95.

For some time now there has been a clear need for a book which presents the findings of the 'archaeoastronomers'—and this means mainly Professor Alexander Thom and his family—on the British prehistoric standing stone sites to the informed general public (an audience which, in this particular case, includes much of the archaeological profession). Thom's discoveries are among the most important and controversial to have confronted prehistorians for many decades; they concern the intellectual qualities which, he claims, lie behind the design and positioning of the stone circles and standing stones and are of a kind which, if accepted, must completely alter our picture of Neolithic British society. Thom's work is, however, couched in highly technical and mathematical terms and can be hard going even for those reasonably competent numerically—though the rewards for perseverance are great.

Dr Wood has succeeded admirably in his task. Here is a lucid, carefully composed and balanced account of the findings of the archaeoastronomical school which makes even the most difficult of these ideas reasonably plain. After reading chapter 7 the reviewer found himself for the first time—after eleven years of experience of this field—with a fair grasp of Thom's theory of the use of the fan-shaped stone rows. This is that they were ancillary apparatus at some lunar observatories which could be used to determine the moon's extreme monthly declinations by extrapolation from the actual observations.

Dr Wood has many sensible things to say about the fallacy of equating the modern skills needed to unravel traces of ancient geometry and astronomy with the prehistoric skills involved. As he says, twentieth century man easily forgets how simple and effective the techniques of the pre-scientific age can be and tends to assume that the ancients must have been phenomenally wise to have done with Stone Age equipment what we use theodolites and electronic calculators to do. In its extreme form, this false premise leads

to the belief that all powerful extra-terrestrials must have been involved. And a milder form of this naive belief leads even some archaeologists to maintain that Thom's deductions are simply not credible because Neolithic man could not have possessed the advanced mathematical and astronomical knowledge that he possesses. Of course they did not, but the same results could still have been achieved from a much simpler technological and intellectual base.

Particularly useful is Dr Wood's careful description in chapter 3 of the methods by which the various geometrical shapes Thom believes were incorporated in stone circle designs—true circles, ellipses, flattened circles and egg-shapes—could have been laid out on the ground using only simple equipment like loops of rope, pegs and measuring rods. This exercise provides a further illustration of why one should not attribute too much abstract knowledge to the megalith builders.

There is little doubt that right-angled triangles were the basis of many of these geometrical shapes and it is easy to conclude that the attraction to the circle-builders of the perfect ones found—the 3:4:5, 5:12:13 and 8:15:17 triangles—was the relationship between the squares on their sides thought to have been discovered by Pythagoras in the sixth century BC.

The idea that the Neolithic circle-builders of Britain anticipated Pythagoras by two millennia or more is compelling and would, if true, considerably alter our ideas on the history of intellectual thought. Dr Wood points out, however, that the fact that some not quite perfect triangles were used—such as $8^2 + 9^2 \approx 12^2$ (actually 12.04)—almost certainly means that these numbers were used because they gave 90° angles when laid out on the ground. We certainly cannot assume that the circle-builders also knew the Pythagorean theorem.

Dr Wood brings the same lucidity and common sense to his review of the astronomical qualities claimed to exist in the standing stone sites and gives us, for example, a useful and up-to-date account of the various theories current about how Stonehenge might have been used as an observatory. Anyone wanting a reliable guide through what sometimes seems like a

maze of theory and speculation about this site cannot do better than this.

Prehistorians will not be satisfied with the relatively meagre amount of space given to the more traditional kinds of archaeological evidence and some will feel that their objections to the ideas of the archaeoastronomical school are not adequately covered (though it is true that little of this is actually in print).

Dr Wood is clearly much more at home with the technical and mathematical evidence than with the historical and archaeological. This shows, for example, in his discussion of the concept of the prehistoric unit of length termed the Megalithic Yard; the statistical uncertainties sway him against the idea but the parallel, historical evidence published by the reviewer—which provides striking independent support for the existence of this unit—is not mentioned. However, the book is intended primarily to be a guide through the complexities of archaeoastronomy and archaeogeometry and it succeeds extraordinarily well. □

Euan MacKie is at the Hunterian Museum, University of Glasgow, UK.

Catastrophe theory

Catastrophe Theory and Its Applications Surveys and Reference Works in Mathematics. By T. Poston and I. Stewart. Pp.491. (Pitman: London, 1978.) £25.

HERE at last is the first connected account of catastrophe theory written for people who are not pure mathematicians but who want to understand the applications of this new branch of geometry. Previously there has been only Thom's classic *Structural Stability and Morphogenesis*, often as deeply obscure as it is stimulating, and Zeeman's *Collected Papers 1972-1977*, often brilliantly clear but necessarily disconnected.

The first half of Poston and Stewart's book presents the mathematics of catastrophe theory. They explain clearly Thom's listing of the ways in which critical points of functions (that

is, maxima, minima and saddles) of n variables can coalesce as k parameters vary. The central classification theorem is not proved, but the main ingredients of the proof (transversality, stability, and so on) are very fully described, to the point at which it becomes clear why the catastrophe hierarchy depends strongly on k but hardly at all on n . In applications one often encounters functions that are not in the form of one of Thom's standard polynomials; then intricate rules determine which catastrophe is involved. It is one of the pleasant features of this book that a whole chapter is devoted to these. The interplay between algebraic and geometric ideas is emphasised throughout with the aid of a large number of diagrams. Functions are considered in the modern way, as mappings between sets, and the corresponding notation is used abundantly. However, it is explained at the beginning, and there is sufficient reversion to the old-fashioned " $f(x)$ " for traditionally educated scientists not to lose the thread of the argument.

The second half of the book describes some applications. These are

mostly in the physical sciences, so that it is possible to state definitely what 'potential function' it is whose coalescing critical points give the catastrophes. In Zeeman's pretty theory of ship stability, for example, the potential is gravitational energy; in optical caustics it is the ray transit time (more generally, the action); in beam buckling problems it is elastic energy; in fluid flow it is the stream function; and in phase transitions it is the Helmholtz free energy. Usually the fold and cusp yield little not already known to specialists in these various fields; only with the swallowtail, umbilics and higher catastrophes does one start to generate surprising richness of behaviour and genuine applications to the theory as opposed to mere illustrations.

These authors are masters of advanced exposition, and they are to be congratulated on producing what I am sure will become a classic in its subject. The publishers deserve credit for the high standard of presentation, but not for the price

Michael Berry

Michael Berry is Reader in Physics at the University of Bristol, UK.

Molecular biochemistry

Electrical Interactions in Molecular Biophysics. By R. Gabler, Pp. 352. (Academic: New York, San Francisco and London; 1978.) \$25; £16.25.

This text forms a very readable handbook which outlines the fundamentals of electrostatic and dielectric theory on the one hand and of chemical bonding and molecular biochemistry on the other. From the beginning, the chemistry is given in terms of organic macromolecules. The author stresses the electrostatic nature of molecular interactions and takes care to develop the concepts of dipole moments and dielectric constants.

A good chapter uses these foundations for a detailed account of the various types of charge and dipolar interactions encountered in real molecules together with some idea of their relative importance. Even though the author uses tables to full advantage in attempts to give the reader a feel for the magnitudes of dipole moments, dielectric constants and polarisabilities in real bonds and molecules, the book lacks the ability to convey confidence and insight as to how the reader should treat, evaluate and really understand the complicated behaviour of say the nucleic acids, globular proteins or poly-

saccharides, for example. Three good chapters form condensed, readable but detailed resumés of Van der Waals forces, Debye-Hückel theory and the structure of water. Emphasis for these is again placed on biopolymer materials.

The final chapter on experimental electrical techniques is somewhat dis-

appointing, however, in that it deals almost exclusively with electrophoretic methods. Nevertheless, this particular method is described very well. It is less fortunate that, even though the measurement of dielectric constants and dielectric dispersion data feature strongly in the book, they are tucked away in earlier theory chapters rather than in the experimental chapter. Other novel methods such as ion exchange chromatography, isoelectric focusing and precipitation get but brief mentions, and electro-optic phenomena are by-passed altogether.

In conclusion, the topics with which the book deals are treated well. The book provides a very readable and concise account of the electrical and interaction phenomena encountered in biopolymer solutions and makes a valiant attempt to link the fundamentals of electrostatics and biochemistry. However, it is perhaps a pity that, after all the theoretical and conceptual material, the author did not give examples of how the experimental data could be analysed, interpreted and related to the basic properties and functions of macromolecules. A very good handbook for graduate students and researchers in the field of molecular biochemistry in general and for those engaged in electrical or dielectric measurements in particular.

B. Jennings

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British bryophytes

The Moss Flora of Britain and Ireland. By A. J. E. Smith. Pp. 706 (Cambridge University Press: Cambridge and London, 1978.) £27.50.

FIFTY-FOUR years have elapsed since the publication of a comprehensive account of British bryophytes. Smith's new book is a worthy replacement for Dixon's *Handbook*, as it combines succinct detail of text with splendid illustrations. It is easier to use than the old *Handbook*, for illustrations are scattered throughout the text rather than being grouped at the end. In many respects it matches the more recent and most popular guide to mosses by E. V. Watson (*British Mosses and Liverworts*; Cambridge University Press second edition, 1968) but surpasses this book in its comprehensive coverage; 692 species are described

and illustrated. It lacks the habit drawings, which were a valuable feature of Watson's book, especially for beginners, but this is not a serious deficiency.

Profuse taxonomic revisions have occurred since Dixon's *Handbook* and the new names will undoubtedly find more widespread acceptance as a result of this publication. *Mnium* now proliferates into *Plagiomnium* and *Rhizomnium*. *Acrocladium* is now *Calliergon* (though we are spared *Calliergonella cuspidata*), and so on. The treatment of that awkward genus *Sphagnum* (contributed by M. O. Hill) is excellent.

This is a book which will be greeted enthusiastically by amateur and professional botanists alike. The price is high but this should not deter the enthusiast; it is worth every penny.

Peter D. Moore

Peter D. Moore is Senior Lecturer in Plant Sciences at King's College, University of London, UK.



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Potato research

The Potato Crop: The Scientific Basis for Improvement. Edited by P. M. Harris Pp. 730 (Chapman and Hall, London; 1978.) £25

THIS book is one of the first of a series of crop monographs; those on barley and potatoes have appeared and one on wheat is promised. According to the general editor of the series, E H Roberts, the aim is to provide authoritative and comprehensive summaries of current scientific knowledge aimed at teachers, students and research and advisory workers (they are not meant as practical manuals for farmers). The project as a whole is timely because the past 20 years has seen a formidable growth of knowledge of the scientific aspects of all our crops, yet there are few good recent monographs of temperate crops available, tropical crops, perhaps surprisingly, have been, on the whole, rather better served.

To what extent, then, does the present work fulfil these intentions? In general, I think it does so very

well. The book, a large one, is composed of 17 chapters which start with history and biosystematics, and go on through morphology, growth physiology, mineral nutrition, water relations, plant density and 'seed' management to mechanisation and weed control; there follow treatments of diseases, pests, tuber quality, storage, breeding, and economics, and the book concludes with a somewhat more speculative (and very interesting) chapter on the application of physiological ideas to the enhancement of crop performance.

The editor has done a good job of making a reasonably coherent whole out of diverse material contributed by 21 authors. The book is well made, the illustrations and index are adequate, and bibliographies (chapter-by-chapter) seem to be fairly complete, misprints and errors are few. Individually, the writers clearly know their subjects well, so the work as a whole is authoritative as well as comprehensive. The facts that our knowledge has improved greatly in recent decades and that the management of the crop and the product is shifting rather rapidly as new technology emerges become very apparent on reading this book. That knowledge and management are both yet imperfect, however, is also clear

and several authors draw attention to deficiencies in these respects.

This book will surely become the standard reference work for the crop and anyone seriously concerned with potato research will need to have it near to hand. Teachers will find it useful and so, perhaps, will some potato farmers (who tend to be knowledgeable enthusiasts); but it is not a practical manual.

There are two defects which should be considered in preparing a revision. First, few readers will find 30 odd pages of taxonomy of wild species very useful in the crop physiological context of this book, and the first two chapters could therefore be condensed into one without loss of content but with some saving of space. Second, though the bibliographies are reasonably international in character, the more agricultural parts of the text are much coloured by Western European, especially UK, practice. A wider viewpoint (including the place and potential of the crop at low latitudes) would be desirable.

N. W. Simmonds

N. W. Simmonds is Lecturer in the School of Agriculture at the University of Edinburgh, UK.

Quantum electrodynamics textbook

Quantum Electrodynamics. By S. N. Gupta. Pp. 226 (Gordon and Breach, New York, Paris and London, 1978.) £25 30.

QUANTUM electrodynamics is the relativistic quantum theory of photons interacting with electrons and other charged leptons. It grew between about 1930 and 1950, as the natural result of combining special relativity with quantum theory. It has made some spectacularly accurate predictions, and it is one of the triumphs of mid-twentieth century physics.

There are few textbooks on just this subject, and the appearance of a new one is noteworthy, particularly when it is by someone like Professor Gupta, who has been a frequent and important contributor to the subject.

One of the best things about this book is its conciseness. Everything is done as economically and elegantly as possible, with no superfluous words. To achieve this compression some things inevitably have had to be sacrificed. I missed any discussion of polarisation,

of bound states, and, most seriously, of the experimental confirmation of the subject. There is a paucity of references, and there are none at all to other textbooks.

On technical matters, the gauge condition is dealt with using the indefinite metric, and regularisation is treated in terms of auxiliary fields. Scattering theory is treated rather perfunctorily. The difficult subject of renormalisation is briefly but, to my taste, sensibly and adequately covered.

Quantum electrodynamics is not a self-contained subject. In calculating the magnetic moment of the electron, for example, weak interactions and the virtual production of strongly interacting particles would have to be included if sufficient accuracy were required. Professor Gupta's book may be one of the last to confine itself to electrodynamics. It is none the worse for that; but a discussion would have been welcome of the place of quantum electrodynamics within modern physics.

This brief and clear book would be a good one from which to learn the subject. It is a pity that the price is too high for most students to pay.

J. C. Taylor

J. C. Taylor is Reader in Theoretical Physics at the University of Oxford, UK.

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obituary

R. G. W. Norrish, 1897-1978

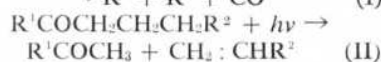
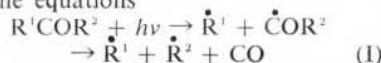
THE death of Professor Ronald George Wreyford Norrish in his 81st year at Cambridge on 7 June 1978 marks the passing of an era notable for remarkable developments in chemistry, among which must be included the advance to maturity of reaction kinetics as a discipline in physical chemistry. Throughout Norrish's research career, extending over more than forty years, he was intimately concerned with a wide variety of subjects, including photochemistry, atomic and free-radical reactions, chain processes such as ignition and combustion and polymerisation, and energy transfer between and within molecules. In all these his studies led to important, and sometimes revolutionary, advances. Wide as his research activities were, yet they were intertwined, with the thread of an intense interest in the behaviour of activated species and the study of the latter by kinetic techniques, running through them.

Norrish's first photochemical work, carried out in 1923 in collaboration with E. K. Rideal, was a study of the photochemistry of aqueous potassium permanganate solutions. At that time photochemistry was in its infancy. The first application of quantum theory to photochemistry had been made by Einstein in 1912 in the form of his law of photochemical equivalence (although in some respects this was anticipated by work of Stark) and its precise applicability to photochemical reactions was still a matter for debate. The remarkable paper by Franck on the elementary processes of photochemical reactions did not appear until 1925. Norrish published a number of interesting papers before 1930, notably those on the photochemistry of nitrogen dioxide, in which he demonstrated a photochemical threshold and the achievement of a photostationary state.

In the period up to the middle 1930s correlation between photodecomposition and photophysical phenomena, such as spectral character and the appearance of fluorescence, engaged the attention of photochemists and Norrish's laboratory in Cambridge occupied a prominent position in these studies. Of his photochemical work before World War II, his long series of investigations of the photolysis of carbonyl compounds is perhaps particu-

larly far-reaching.

The first paper, in 1928, concerned with the photodecomposition of glyoxal, was followed by reports of studies of formaldehyde, acetaldehyde, acetone and a variety of aldehydes and ketones carrying longer hydrocarbon chains. These led to the recognition of the now famous Norrish Type I and Norrish Type II reactions, illustrated by the equations



in which the groups R^1 and R^2 are understood to contain one or two carbon atoms. The observation that Types I and II reactions are influenced strongly by the physical environment—i.e. whether gas phase or condensed phase—provided a most important 'spin-off', namely the first experimental illustration of the Franck-Rabinowitch cage mechanism operative in solution.

Norrish's interest in intense light sources for photolysis seems to have arisen during World War II. I recall a comment (suitably embellished), made near the end of the war, that he intended to get a searchlight to photolyse keten and to trap the resulting methylene by the Paneth method. At that time, rudimentary ideas of flash photolysis may have been growing in his mind.

Flash photolysis, pioneered by Norrish and Porter and their coworkers, was a truly revolutionary development. It makes use of a powerful short-lived 'photoflash' which brings about rapid dissociation of the absorbing species into radicals or atoms. A second weaker spectroscopic flash ('specflash') triggered electronically at a precise interval after the first, permits the observation of transient species and by varying the time between the two flashes the kinetics of the formation and decay of such species may be studied. A fascinating account of these developments, extending from pyrolysis and combustion processes to vibrational relaxation phenomena, was given by Norrish in his Faraday Lecture to the Chemical Society. The immense impact of this technique on the study of fast reactions and the behaviour of active species is now too well-known to require description and the award of the



Nobel Prize for Chemistry in 1967 jointly to Norrish and Porter and to Eigen was universally welcomed.

Norrish's interest in chain processes was evident from his early work on the hydrogen-chlorine reaction in which he elucidated the inhibitory effects of oxygen and ammonia. Adoption of a new technique (continuous photometric analysis) enabled Norrish and Ritchie to establish that the retarding effect of HCl in pure systems and systems containing oxygen is attributable to the process



Norrish's work on the kinetics of this chain reaction, many features of which had defied solution by earlier workers, established his eminent position among kineticists.

Much of Norrish's work on combustion was centred on the transition between slow reaction and ignition and particularly the dependence of the ignition boundaries on the concentrations of various additives (e.g. NO_2 , NOCl , CCl_3NO_2). During the early stages, Semenov was developing his classical theories of thermal and isothermal ignition; Norrish saw the necessity for combining both these in a branched-chain thermal theory. Although some of the detailed interpretations have been modified as the result of applications of new experimental techniques, there is no doubt that the beautiful work of Norrish and his colleagues, made over a long period, has provided much of the basis of our present understanding of these complex processes. The experimental demonstration of degenerate (or delayed) branching in hydrocarbon combustion was an outstanding contribution.

Royal Society of Medicine
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Number 1

Piroxicam

edited by W. M. O'Brien and E. H. Wiseman

August 1978, viii + 112pp., £5.00/\$10.25 0.12.523850.9
Proceedings of a symposium organised by Pfizer Central Research
Division in conjunction with the American Rheumatism
Association Section of the Arthritis Foundation during the XIVth
International Congress of Rheumatology, San Francisco,
California, USA, on June 30, 1977.

Published jointly by The Royal Society of Medicine, 1 Wimpole Street, London
Academic Press London and Grune & Stratton, New York

Schizophrenia: Towards a New Synthesis

edited by J. K. Wing

October/November 1978, xii + 292pp., £8.00/\$16.50 0.12.759450.7

Advances in the understanding of schizophrenia have lately been made in the teeth of controversy and confusion, part of which is due to differences in terminology or diagnostic criteria, and part to the elusive nature of the condition itself. It seems clear that the truth about schizophrenia is not to be found within one restricted field of knowledge. At last, however, the beginnings of a convincing synthesis are appearing, balancing biochemical and genetic evidence with findings from clinical psychiatry and the behavioural sciences, establishing a basic set of symptoms and disposing once and for all of the argument that schizophrenia is a myth. This book provides a comprehensive and readable account of modern knowledge about schizophrenia.

Published in New York by Grune & Stratton Inc. (ISBN 0.8089.1140.6)

Chemisorption of Gases on Metals

F. C. Tompkins

October/November 1978, x + 372pp., £16.80/\$34.75 0.12.894650.7

This volume will fill the gap in providing an up-to-date account of chemisorption on metals for the graduate and postgraduate student and specialist, in forming a basis for further studies. The early part of the book introduces the thermodynamics and statistical mechanics of chemisorption; but subsequent chapters on the kinetics of absorption and desorption processes place their emphasis on recent investigations of sticking probabilities, precursor and multiplet states, energy accommodation and molecular beam scattering. Later chapters are concerned with the geometric and electronic properties of surfaces and their overlayers, and include adsorption-reflection infra-red spectrometry, LEED, Auger, ultra-violet and X-ray photoelectron spectroscopy, ion neutralization and energy loss spectroscopy, elastic resonance and inelastic tunnelling in field emission. A brief account of the status of modern theoretical studies is also included.

Techniques of Physics Series

Series editors: N. H. March and H. N. Daglish

Digital Filters and Their Applications

V. Cappellini, A. G. Constantinides and P. Emilian

October/November 1978, xvi + 384pp., £20.50/\$42.40 0.12.159250.2

Over the last decade, there have been remarkable developments in sampled data processing methods and techniques, the most important of which is digital filtering. These developments have resulted in many new and useful applications for digital filters in areas such as communications, radar-sonar, experimental physics, aerospace systems, biomedicine and remote sensing. This book presents a synthesis of the theory and design of digital filters (1-D and 2-D) together with a description of their practical utilization in the most important areas of signal and image processing. The book is divided into three main parts. The first, tutorial in character, summarizes the basic relationships for the different types of digital filter, and presents design methods. The second tackles practical problems of design and implementation, and the third describes the impact of digital filters in research, operative and industrial areas.

Active and Passive Thin Film Devices

edited by T. J. Courts

October/November 1978, xxiv + 858pp., £60.00/\$124.25 0.12.193850.8

In recent years both the technology and the basic understanding of thin film phenomena have advanced rapidly. Although few new passive thin film devices have been developed, the materials used have become increasingly well understood, with better knowledge of their properties, and at the same time developments have been made in the theories of charge transfer. The rate of development of active devices, however, is very rapid and it seems certain that in the very near future these will have a major impact on many fields including information display, communications, energy conversion and detection, medicine, computing and instrumentation. There is therefore a great need for a book such as this which provides a comprehensive account of the properties of thin films, the theories of thin film phenomena and their wide range of applications.

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announcing volume 3

Development in Mammals

Development in Mammals is an advanced, critical and informative work of reference which serves not only to record the current states of knowledge and controversies in the field of mammalian development but also point the direction of new approaches. Authors contributing to this series have been encouraged to extend their observations beyond the narrow range of mammalian development wherever interdisciplinary approaches or parallels drawn with other areas might appear useful.

edited by MARTIN H. JOHNSON

Volume 3

1978. viii + 472 pages. US \$ 61.00/
Dfl. 140.00. ISBN 0-7204-0663-3

Volume 3 of *Development in Mammals* shows some alterations in style, but has adopted the same approach to content as did volumes one and two. Authors have again been encouraged to develop new ideas and to be provocative, and to use unpublished data where these would support an argument. Within this flexible framework, a number of contributions have been received relating to four broad categories of topic.

Two of the contributions deal with the acrosome reaction, one approach biochemical and the other biophysical. Two contributions are concerned specifically with models for cell:cell interaction in development, either by viral particles or through localised fluidity changes in cell membranes. Five contributions deal with the relationships between stem cells, pluripotentiality and the transmission of the germ line. Finally, three contributions are concerned with the fundamental aspects of morphogenesis - proliferation, migration and clonal growth.

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Nature

1977. viii + 242 pages. US \$31.50/
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In the field of polymerisation, Norrish also made pioneering kinetic studies. The best-known is probably that concerned with the acceleration which occurs in the later stages of free-radical polymerisation, to which the name 'gel-effect' has now been attached. Norrish and Smith showed that this is the result of a steadily decreasing rate of chain termination which sets in when the viscosity becomes high. A nice example of the cross-fertilisation of his ideas is provided by his work on the photolysis of poly(methylvinylketone), which was found to undergo photolysis in solution according to both Types I and II. The former yields macroradicals which, in the presence of a polymerisable monomer, give block copolymers.

Norrish had intense energy and enthusiasm and an all-consuming desire to get to the bottom of things by experimental means. These qualities could be disconcerting to his research students, who were sometimes expected to obtain definitive results between 9.30 a.m. and 6.0 p.m. on the same day (bank-holidays included!). Norrish was a generous and warm-hearted, yet forthright, person. His membership of the Savage Club reflected his enjoyment of meeting people, especially those distinguished in professions remote from his own. He had a tremendous zest for life and his hospitality became legendary, not only on the more formal occasions, but also in his entertainment of friends and colleagues from all parts of the world at his home in Park Terrace. There, one might be regaled with recordings of Japanese music or a lecture by Rutherford, or enter into quasi-philosophical discussions of the 'fourth law' of thermodynamics and when Norrish received his honorary degree in the University of Liverpool, the Public Orator recalled the remarkable aptness of Praed's words

'Beginning with the laws which keep
The planets in their radiant courses,
And ending with some precept deep
For dressing cels, or shoeing horses.'
It might truly be said that with Norrish every evening was Christmas eve.

Things Cantabrigian occupied a very special place in Norrish's affections. He was born and educated in Cambridge and effectively spent all his working life there. In 1937, after the death of T. M. Lowry, he had become the second holder of the Chair of Physical Chemistry and subsequently the head of the separate Department of Physical Chemistry. He was justifiably proud of the department he had built up and had strong associations with his College (Emmanuel) of which he was Senior Fellow at the time of his death. His lectures on physical chemistry were

often vividly illustrated by spectacular demonstrations of his favourite phenomena in photochemistry and chain processes. During the war, his department was largely concerned with problems related to the war-effort, such as suppression of gun flash and the development of incendiary materials; during this time Norrish was Chairman of the Incendiary Projectiles Committee.

Norrish's distinction naturally led to his receiving many honours. He was elected Fellow of the Royal Society in 1937 and received honorary doctorates from the Sorbonne, and the Universities of Lancaster, Leeds, Liverpool, Sheffield and British Columbia. He was awarded the Meldola Medal of the Institute of Chemistry (1928), the Davy Medal of the Royal Society and the Liversidge Medal of the Chemical Society (1958), the Lewis Gold Medal of the Combustion Institute (1964), the Faraday and Longstaff Medals of the Chemical Society (1964, 1968) and was a member of eight foreign Academies of Science. During 1953-55 he was President of the Faraday Society, and in 1961 was President of Section B of the British Association for the Advancement of Science. In 1966 he gave the Bakerian Lecture of the Royal Society. Notwithstanding all his honours, he retained an innate modesty which allowed him to accord to others, particularly his junior colleagues, due appreciation of their efforts.

In 1926 he married Anne, the eldest daughter of A. E. Smith, of Manchester, who survives him. They had twin daughters.

C. H. Bamford

J. P. Kendall

PROFESSOR JAMES PICKERING KENDALL, F.R.S., F.R.S.E., Emeritus Professor of Chemistry, University of Edinburgh, died in Edinburgh on 14 June 1978.

He was born on 30 July 1889 in Chobham, Surrey, and was educated at Farnham Grammar School and Edinburgh University. A distinguished undergraduate career was followed by work on exact conductivity measurements, first in Edinburgh and later with Svante Arrhenius at the Nobel Institute, Stockholm. Kendall was inspired by Arrhenius, but found research facilities in the Institute disappointing and after a spell he went to the better equipped Technological Institute of St. Petersburg.

In 1913 Kendall went to Columbia University, New York, as an instructor in chemistry and in 1922 was promoted to a full professorship. He carried out research on a variety of topics includ-

ing the formation of addition compounds in solution and ionisation equilibria. A good example of his inclusive and discriminating approach to research is contained in a long, critical and polemical paper on the properties of strong electrolytes in solution in which facts and arguments are marshalled against the views and postulates of Ghosh.

Probably his most important work was the attempt to separate isotopes and mixtures of rare earths, a pioneer effort at a period when the sophisticated armoury of the modern chemist was not available. Isotopic separation eluded him, but by the ionic migration method he effected a substantially complete separation of yttrium from erbium. At the same time he undertook the revision of Alexander Smith's famous series of chemistry textbooks, a task which made increasing demands on his time and energy.

In 1927 Kendall was elected a Fellow of the Royal Society and a year later succeeded Sir James Walker as Professor of Chemistry in the University of Edinburgh, a post he held until his retirement in 1958. He immediately made full use of his administrative ability and business acumen into extending the research facilities of his department, and with the help of men like J. A. V. Butler, E. B. Ludlam, M. Ritchie and H. W. Melville, succeeded in building up a thriving school of physical chemistry. Apart from unsuccessful attempts to separate the hydrogen isotopes in water and to detect the calcium isotope with mass 41 in the potassium-rich deposits of Rhiconich in Sutherlandshire Kendall gave up research and concentrated on writing books. These included *At Home among the Atoms* and *Young Chemists and Great Discoveries* and the lively and readable biographies of Michael Faraday and Humphry Davy. In 1938 he wrote *Breathe Freely* in which he correctly predicted that in the event of war poison gas would not be used.

Kendall was an excellent lecturer and he gave the 1938/39 course of Christmas lectures at the Royal Institution, in London. In one of the lectures he performed the famous boiling lead experiment, when his daughter Jean passed her hand through the stream of white hot metal. He also took a great interest in the Royal Society of Edinburgh and served first as General Secretary (1936-1946) and then as President (1949-1954). In his younger days he enjoyed bicycling and he was a very good bridge player. He was twice married and his second wife, Jean, tended him devotedly in his latter years of ill-health. He leaves a son and two daughters.

Neil Campbell

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Applications are invited from experienced scientific officers for a post which is available immediately. Duties include screening and identifying HLA antibodies, typing of patients and potential donors for the bone marrow transplantation programme, participation in regional and international workshop exchanges of anti and information. New developments include the identification of B antibodies and the application of studies in malignant diseases.

Application Forms from Person Dept. Tel. 352 8171 Ext. 447. Clo date: September 17, 1978. 6078(A)

LABORATORY SCIENTIFIC OFFICER required to work in Department of Histopathology. Anatomy for three years to assist work on the immunology of multiple sclerosis and cell surface antigens of the nervous system. Experience of cells/tissue culture an advantage. Salary for qualified Laboratory Scientific Officer not less than £3,261 £354 London Weighting. Apply writing, stating age and giving details of qualifications and experience quoting the names of two referees to the Secretary, Guy's Hospital Medical School, London SE1 9RT. Additional information obtainable from Leibowitz, Department of Histopathology (Tel. 01-407 7600, ext. 33 6077(A)

UNIVERSITY OF ABERDEEN DEPARTMENT OF NATURAL PHILOSOPHY SENIOR LECTURESHIP

Applications are invited for Senior Lectureship in the Department of Natural Philosophy from candidates well qualified for research and university teaching in theoretical physics. The successful applicant is expected to take up his or her duties as soon as convenient and later than October 1, 1979.

Salary on scale £7,074 to £8,000 per annum, with appropriate place. Further particulars from Secretary, The University, Aberdeen with whom applications (2 copies) should be lodged by October 1, 1978. 6093(A)

ASSISTANT PROFESSOR BOTANY

The Department of Biology, University of Virginia is seeking candidates in the areas of molecular cellular or developmental botany an assistant professorship for September 1979. Postdoctoral experience highly desirable. By November 1, 1978 candidates should send a curriculum vitae and summary of research plan and have three letters of recommendation sent to: L. L. Rebbun, Faculty Search Committee, Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, Va 229. An Affirmative Action/Equal Opportunity Employer 6094(A)

MEDICAL RESEARCH COUNCIL STATISTICIAN

The M.R.C. Clinical and Population Cytogenetics Unit, Edinburgh has an opening for a statistician with a Ph.D. or a good Honours Degree and equivalent research experience. The Unit offers extensive and varied opportunities for the application of statistical methods to the design and analysis of experiments; and in demography.

The appointment is expected to be limited to a three year term, on scale £4,365 to £5,730, though of types of contract may be considered for exceptionally well-established research statisticians.

To apply, or for further information please contact in the first instance Administrative Officer, M.R.C. Cytogenetics Unit, Crewe Road, ED BURG 4. Closing date for applications September 30, 1978. 6095(A)

**THE QUEEN'S
UNIVERSITY OF
BELFAST
POSTDOCTORAL
RESEARCH ASSISTANT
DEPARTMENT OF APPLIED
MATHEMATICS AND
THEORETICAL PHYSICS**

A postdoctoral research assistant is required to work on the theory of resonant multiphoton processes (particularly multiphoton ionisation) with Dr S. Swain. The basic requirement is a good working knowledge of quantum mechanics; experience in quantum optics would be an advantage but is not essential.

The appointment which is funded by the S.R.C. is for a period of twelve months at a salary of £3,660 (with U.S.S.).

Curriculum vitae and the names and addresses of two referees should be sent as soon as possible to the Personnel Officer, The Queen's University of Belfast BT7 1NN, N. Ireland. 7043(A)

**UNIVERSITY OF
LIVERPOOL
DEPARTMENT OF
INORGANIC, PHYSICAL AND
INDUSTRIAL CHEMISTRY
POST-DOCTORAL
RESEARCH IN POLYMER
SCIENCE**

Applications are invited for the post of Senior Research Assistant, to work with Dr G. C. Eastmond on the dynamic-mechanical properties of multicomponent polymers. Work will involve installation of equipment, establishment of experimental techniques and application to the study of molecular motions in multicomponent polymers of known chemical structure and morphology. Applicants should have relevant experience.

The post, tenable immediately, is available for a maximum period of two years. The starting salary will be £3,883 or £4,133 per annum plus U.S.S. benefits.

Applications, together with the names of two referees, should be received not later than September 29, 1978, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/921/N. 7049(A)

**MEDICAL RESEARCH
COUNCIL
TOXICOLOGY UNIT
PHARMACOLOGIST/
PHYSIOLOGIST**

required as part of a long-term programme to build up expertise for studies of the mechanism of action of toxic substances on specific organs or preparations *in vivo* and *in vitro*. He will have opportunities for interdisciplinary studies with other members of the staff as well as detailed discussions with Professor E. Zaimis of the Department of Pharmacology, Royal Free Hospital Medical School. The post will be a three-year post-doctoral temporary appointment.

Apply in writing by the end of September enclosing a full curriculum vitae to Dr W. N. Aldridge, Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF. 7048(A)

**ASSISTANT PROFESSOR
BOTANY**

The Department of Biology, University of Virginia is seeking candidates in the areas of molecular, cellular or developmental botany for an assistant professorship for September 1979. Postdoctoral experience is highly desirable. By November 1, 1978, candidates should send a curriculum vitae and summary of research plans and have three letters of recommendation sent to: L. I. Rebhun, Faculty Search Committee, Department of Biology, Gilmer Hall, University of Virginia, Charlottesville, Va. 22901. An Affirmative Action/Equal Opportunity Employer. 6094

**CSIRO
AUSTRALIA**

**Research Scientist or
Postdoctoral Research Fellow
Australian Numerical Meteorology
Research Centre Melbourne VIC**

CSIRO has a broad charter for research into primary and secondary industry areas. The Organisation has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field:

Tropical Meteorology/Dynamic Meteorology.

General:

The Australian Numerical Meteorology Research Centre is a joint venture of the CSIRO and the Department of Science (Bureau of Meteorology). The Centre's research consists of studies of the behaviour of the earth's atmosphere, with emphasis on general circulation, directed towards improvement in the accuracy and time scale of weather forecasting, and towards improvement in understanding the distribution and variations in climate on the earth.

In this context the Centre is especially interested in developing an improved understanding of the physical mechanisms associated with interannual variability and particularly the formation and maintenance of droughts, and has an established modelling commitment directed towards this goal. The Centre is anxious to increase this commitment through the appointment of an additional scientist specialising in either the modelling of interannual variations of the large scale global circulation, or the special role of low latitude processes as a determining factor in climate variations. Access is available to the computing facilities of both the CSIRO and the Bureau of Meteorology, and a variety of large scale models developed at the Centre.

Duties:

To conduct appropriate experiments with atmospheric models consistent with the Centre's research goals, and to develop improved models suitable for studying seasonal variations of the large scale global circulation.

or

To assess through a combination of modelling experiments and observational studies the role of low latitude processes in determining variations in the large scale atmospheric circulation.

Qualifications:

Applicants should have a Ph.D. degree relating preferably to tropical meteorology or dynamic meteorology or postgraduate experience of equivalent standard and duration, supported by satisfactory evidence of research ability.

Salary:

Research Scientist: \$A14,829-\$A18,177 p.a. or Senior Research Scientist: \$A18,819-\$A21,543 p.a. Scientists with considerable experience and outstanding ability should not be deterred from applying as appointment at a higher level and salary would be considered.

Tenure:

Either: (i) an indefinite appointment as Research Scientist
or (ii) a fixed-term appointment as Post-Doctoral Research Fellow, may be negotiated. Both carry Superannuation benefits.

Applications *IN DUPLICATE*, stating *FULL* personal and professional details, the names and addresses of at least two professional referees, and *QUOTING REFERENCE NUMBER 424/28* should reach The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON, WC2R 3EH by October 7 1978.

Applications in U.S.A. and Canada should be sent to: The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C., 20036, U.S.A. 7016(A)

Nature Conservancy Council (Commissioned Research - 2 year posts) Scientific Officers (8) and Higher Scientific Officers (2)

Salary: Scientific Officer £2,839*

Higher Scientific Officer £4,101

*The Officers based in London will also qualify for Inner London Weighting, ie, £485 per annum.

Applications are invited for the following contract posts in the Nature Conservancy Council, each for a fixed period of two years.

1) Invertebrate Surveys of (a) Somerset Levels and (b) Marshes in Kent and Sussex

Two survey teams are to be set up, one for each of the above areas. Each team will require two entomologists (1 freshwater and 1 terrestrial) and a botanist; one of the entomologists acting as a Team Leader (HSO) and the other two posts (SOs) in support.

2) Collation and Assessment of Species and Data for Invertebrates

Four Scientific Officers, based in Scotland (Edinburgh), Wales (Bangor) and England (2) (London) are required. Officers will not, however, work in complete isolation, as it will be necessary to have some cross flow of data, etc.

Age and Qualifications: Candidates should have a first or second class honours degree, or equivalent, preferably in botany or zoology.

Scientific Officer: Candidates should be under 27 years of age.

Higher Scientific Officer: Candidates should be under 30 years of age and have at least two years relevant postgraduate experience.

All posts involve a certain amount of travelling, therefore, possession of a current driving licence would be an advantage.

Application forms and further details are available from Miss S Jones, Recruitment Section, Nature Conservancy Council, Godwin House, George Street, Huntingdon, Cambs. PE18 8BG (Tel. 0480 56191). Closing date for completed application forms is 6 October 1978. 6063(A)

SCIENCE RESEARCH COUNCIL

FELLOWSHIPS

and

OVERSEAS (NATO) FELLOWSHIPS AND STUDENTSHIPS

Applications for SRC Fellowships and Overseas (NATO) Fellowships and Studentships commencing in 1979 should be made to the Science Research Council, PO Box 18, Swindon SN2 1ET.

For application forms telephone:

Swindon (0793) 26222, Ext. 2172

Closing date for applications:

Senior Fellowships	: November 30, 1978
Advanced Fellowships	: September 30, 1978
Postdoctoral Fellowships	: January 31, 1979
Industrial Visiting Fellowships	: Apply at any time
Overseas (NATO) Fellowships:	
In Europe	: February 1, 1979 and June 1, 1979
Outside Europe	: January 31, 1979
Overseas (NATO) Studentships	: January 31, 1979

7006(A)

GERMAN CANCER RESEARCH CENTER POSTDOCTORAL RESEARCH ASSISTANT RNA tumor viruses

The work involves studies on the mechanism of transformation by avian leukemia viruses. The salary will be about DM2,900 to DM3,100 per month for an appointment of two years. Send application with curriculum vitae and telephone number and address from two references to Dr Thomas Graf, Institut für Virusforschung, Deutsches Krebsforschungszentrum, Im Neuenheimer Feld 280, 6900 Heidelberg. 7021(A)

THE MACAULAY INSTITUTE FOR SOIL RESEARCH DEPARTMENT OF SOIL SURVEY

Applications are invited for three Soil Survey Officers to work on the Survey of Scotland. Candidates should possess an Honours Degree in Geology, Soil Science, Agriculture, Chemistry or Botany.

The appointment will be in the Scientific Officer grade salary £2,839 to £4,415 per annum, according to qualifications. Non-contributory Superannuation Scheme operative.

For initial training the successful candidate may be posted to various areas in Scotland and thereafter stationed at a decentralised Soil Survey office.

Forms of application and further particulars may be obtained from The Secretary, The Macaulay Institute for Soil Research, Craigiebuckler, Aberdeen AB9 2QJ, to whom they should be returned before September 29, 1978 (Quote Ref. 78/24.25.26). 7047(A)

SALFORD AREA HEALTH AUTHORITY (TEACHING)

HOPE HOSPITAL,
(University of Manchester
School of Medicine),
Eccles Old Road,
SALFORD M6 8HD
RESEARCH
TECHNICIAN

required for the recently formed Department of Rheumatology. The job will involve the general running of the laboratory and assistance with research into immunological aspects of rheumatoid and other inflammatory conditions. Experience in tissue culture and/or immunological techniques would be preferred.

Salary will depend on qualifications and experience and it is hoped that the successful candidate will be appointed on the salary scale £3,261 rising to £4,680.

Application forms and job description can be obtained from the Sector Administrator Tel No 789 7373 Ext. 202 to whom they should be returned by September 15, 1978. 7037(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF MOLECULAR BIOLOGY

Applications are invited for two postdoctoral research positions. The first concerns the biochemical basis of regulation of conjugation in F-like plasmids, and is for a period of from one to three years with a starting date within the period September-December 1978. The second involves work on *Pseudomonas* plasmids and is for a period of one year beginning January 1, 1979. Salaries for both positions will be on Research Range 1A at points commensurate with age and experience.

Applications to, and further details from, Dr N S Willetts, Department of Molecular Biology, University of Edinburgh, Edinburgh EH9 3JR. Please quote Reference 5027 R. 6039(A)

UNIVERSITY OF BRISTOL DEPARTMENT OF PHYSIOLOGY

The Agricultural Research Council has awarded a

RESEARCH ASSISTANTSHIP

to the Department of Physiology for a project concerned with the mechanism of motility in mammalian spermatozoa. The work will depend principally on specialised techniques in light and electron microscopy.

Applications are invited from recent graduates who have a good Honours degree in Physiology or an associated discipline. The salary of the assistant will be on the national salary range 1B, £3,189 to £4,601 (under review). The appointment will be for 3 years and registration for a postgraduate degree will be expected. Applications, together with the names of two referees, should be sent as soon as possible to Dr D M Woolley, Department of Physiology, The Medical School, University of Bristol, Bristol BS8 1TD. 6044(A)

UNIVERSITY OF OTAGO and OTAGO HOSPITAL BOARD Dunedin, New Zealand SENIOR LECTURER IN MEDICINE

(Infectious Diseases) and
CLINICAL MICROBIOLOGY

Applications are invited for the position of Senior Lecturer in Medicine (Infectious Diseases) in the Department of Medicine, University of Otago and Consultant in Infectious Diseases and Clinical Microbiology to the Otago Hospital Board.

This is a full-time University Hospital post with University conditions of appointment and carrying hospital status in the Dunedin Hospitals.

Salary: NZ\$18,022 to \$23,302 per annum inclusive of cost-of-living adjustments. A further adjustment is pending.

To compensate for clinical responsibility, an allowance not exceeding 10% of basic salary may be paid.

Further particulars are available from the Association of Commonwealth Universities (Appa), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University, Box 56, Dunedin.

Applications close on October 31, 1978. 7063(A)

UNIVERSITY OF NATAL DEPARTMENT OF BIOCHEMISTRY Pietermaritzburg

Applications are invited from suitably qualified persons regardless of sex, religion, race, colour or national origin, for appointment to the post of

SENIOR TECHNICIAN

Experience with a range of biochemical techniques such as chromatography, electrophoresis, amino acid analysis, ultracentrifugation or radioisotope techniques, and a knowledge of biochemical instrumentation will be an advantage.

The salary scale attached to the post is:

R5,820 by 240 to R6,300 by 360 to R6,660 plus 15% per cent pensionable allowance per annum.

The commencing salary notch will be dependent on the qualifications and experience of the successful applicant. In addition, an annual vacation savings bonus is payable, subject to Treasury regulations.

Application forms, further particulars of the post and information on pension, medical aid, group insurance, staff bursary, housing loan and subsidy schemes, long leave conditions and travelling expenses on first appointment are obtainable from the Registrar, University of Natal, P.O. Box 375, Pietermaritzburg, 3200, South Africa, with whom applications, on the prescribed form, must be lodged not later than October 20, 1978, quoting reference PMB 34/78. 6067(A)

**IMPERIAL COLLEGE OF
SCIENCE AND
TECHNOLOGY
DEPARTMENT OF
BIOCHEMISTRY**

Applications are invited for two posts in a new, well-equipped research group investigating cell membrane diseases:

**1. RESEARCH
TECHNICIAN
(Grade 3)**

for biochemical investigations of erythrocyte membrane proteins, particularly those affected by hereditary spherocytosis. A sound biochemical background and previous experience in protein chemistry, electro-phoresis and chromatography are desirable. Reference DMS-RT3.

**2. RESEARCH
TECHNICIAN
(Grade 5)**

for studies involving the growth in culture of muscle cells from normal and dystrophic animals, and their preparation for electron microscopic examination by the freeze-fracture technique. Previous experience in tissue culture and/or electron microscopy is highly desirable. Reference DMS-RT5.

The appointments are initially for one year, extendable subject to continuing support from granting agencies. Starting salaries are £3,153 and £3,651 respectively, including London Weighting.

Write or telephone for an application form to the Departmental Superintendent, Biochemistry Department, Imperial College, London SW7 2AZ. (Tel. 01-589 5111 Ext. 1119, quoting the appropriate reference.

6007(A)

**UNIVERSITY OF THE
WEST INDIES—JAMAICA**

Applications are invited for the following posts:

**PROFESSOR/SENIOR
LECTURER IN PHYSICS**

The appointment to be made is in the field of Materials Science. The appointee is expected to continue to shape and teach undergraduate as well as "projected" graduate courses; he/she will also be expected to lead in the further development of research in the area of Materials Science. Industrial experience will be an advantage.

LECTURER IN ZOOLOGY

While applications are invited in any field of Zoology preference may be given to a well-qualified applicant with interests in parasitology.

Salary scales: Professor J\$17,166 to J\$21,251 p.a.; Senior Lecturer J\$12,066 to J\$16,783 p.a. (£1 sterling=J\$2.99). F.S.S.U.; Study and Travel Grant. Unfurnished accommodation will be let by the University at a rental of 10% of salary or a housing allowance of 20% of salary will be paid. Family passages. Detailed applications (2 copies) including a curriculum vitae naming three referees should be sent direct to the Registrar, University of the West Indies, Mona, Kingston 7, Jamaica, as soon as possible. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

7001(A)

RESEARCH ASSISTANT

Graduate required to join a team of scientists investigating basic aspects of cardiac biochemistry and physiology. A training in physiology or biochemistry and experience in spectrophotometry, tissue metabolite analysis or organ perfusion would be advantageous. The research unit is located in the new Rayne Institute and appointments will be made through St Thomas's Hospital Medical School.

Applications together with a curriculum vitae should be sent to Dr D. J. Hearse, The Rayne Institute, St Thomas's Hospital, London SE1. 7031(A)

SCIENCE GRADUATES— A CAREER IN EXPERIMENTAL PHYSICS?

The Vacancy

The Daresbury Laboratory has a vacancy for a young experimental physicist to assist in the preparation of equipment for the synchrotron radiation source which will commence operations during 1980. This preparatory work covers the design of beam lines for both UV and X-ray applications, the development of new instruments for the production of monochromatic radiation and the implementation of experimental programmes by Universities and other research organisations.

The man or woman appointed will be expected to take part in the practical aspects of research and development work under the supervision of experienced scientists and technical staff. A recent graduate with a first or second class honours degree in science or engineering is envisaged for this post. The appointment will be made initially at Scientific Officer level (salary in the range £2,839 - £4,415) according to qualifications and experience.

Background Information

Daresbury Laboratory is situated in a pleasant area of Cheshire and embraces a wide range of scientific disciplines. There is a well established computational science and theory group which interacts strongly with the experimental groups, and thus the person appointed will have every opportunity of developing his/her expertise and knowledge in a variety of subjects. In particular, the Synchrotron Radiation Source will be used by over 100 research teams from UK Universities for experiments in physics, chemistry and biology.

Closing date: 29 September 1978

Please write or telephone Warrington 65000, Ext. 467, for an application form quoting reference number DL/637k to:

**The Personnel Officer
DARESBUURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD**

6071(A)

**GRADUATE OR POSTDOCTORAL
RESEARCH ASSISTANT** required for 1½ years in the Department of Microbiology for an M.R.C. research project on cytomegalovirus immunovirology. Laboratory experience in virology essential.

Applications, with full curriculum vitae and the names of two referees, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. M.C.2. 6076(A)

SENIOR MEDICAL LABORATORY SCIENTIFIC OFFICER

required in Medical Unit for research programme relating to kidney disease and hypertension. Applicants should be State Registered, have a knowledge of techniques used in biochemical and radio-immunological assays and be prepared to undertake biological assays.

Salary within scale £4,347 to £5,769 p.a. plus £354 p.a. London Allowance.

This is a re-advertisement and previous applicants need not re-apply.

Apply in writing with curriculum vitae and two referees to The Secretary, St. Mary's Hospital Medical School, Paddington, London W2 1PG, quoting Ref. N/S/MU. 7066(A)

Pharmacist

**Control of sterile medical products
(up to £7,525)**

The Scientific and Technical Branch of DHSS provides scientific, engineering and other professional services essential to the provision of medical apparatus, instrumentation and supplies to hospitals.

Joining a team which provides advice on, and assesses the safety and quality of, sterile medical products and devices used in the NHS the successful candidate will be concerned with the evaluation and control of sterile medical devices. Assessment covers mechanical performance, good manufacturing practice, sterilization and packaging. The work includes inspecting and advising on the manufacturing facilities in the UK and overseas and involves close liaison with medical and other professional staff in hospitals and industry.

Candidates must have a degree, or an equivalent qualification, in Pharmacy or a closely related field, and have relevant professional experience. A knowledge of plastics and familiarity with the production, assembly and packaging of sterile medical devices advantageous.

Starting salary between £6,200 and £7,525 depending on qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For application form (to be returned by 28 September 1978) write to Civil Service Commission, Alencon Link, Basingstoke, Hants. RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote T/9904/3.

Department of Health and Social Security, London. 6066(A)

Applications are invited for the following post:

FACULTY OF SCIENCE — WEST HAM PRECINCT Department of Paramedical Sciences

LECTURER II IN MICROBIOLOGY WITH SPECIAL INTEREST IN FOOD MICROBIOLOGY

to teach General Microbiology and Food Science or Food Technology or Food Microbiology on the following courses.

- B.Sc. Applied Biology (Sandwich)
- B.Sc. Applied Biology (part-time)
- HNC Applied Biology
- HND Applied Biology
- B.Sc. Life Sciences (part-time)
- Other Special and Short Courses

Research on an individual or group basis will be encouraged and the successful applicant will be expected to contribute to course development.

(Temporary post available vice permanent appointment with effect from 1.9.78) (Ref. S/CF. 142)

Salary Scale:

Lecturer II: £4,101 to £6,558 per annum

Further details and application form from:

The Senior Academic Personnel Officer (2)
North East London Polytechnic
Forest Road
London E17 4JB
Telephone: 01-527 2272 (Extension 20).

Closing date: September 22, 1978.

6081(A)

UNIVERSITY OF RHODESIA CHAIR OF PHYSICS

Applications are invited for the post of Professor and Head of the Department of Physics, which will become vacant on December 31, 1978. Applicants should have held a senior university appointment in Physics and have displayed marked ability in both teaching and research. They should preferably have an interest in geophysics as well as classical physics, since much of the advanced teaching and research undertaken by the Department lies in the former area.

Salary Scale: £10,474 to £394 to £12,444 (Approx. Sig. equiv.).

Appointment will be on permanent, pensionable terms and these include: family passages and allowance towards transport of effects; installation loan of up to half of one year's salary if required; unfurnished University accommodation guaranteed for a period of at least three years for persons recruited from outside Rhodesia; sabbatical leave and biennial visits with travel allowances; superannuation and medical aid schemes.

Applications: (6 copies) giving full details of personal particulars, qualifications, experience, research, publications together with the names and addresses of three referees should be sent to the Assistant Registrar (Science), University of Rhodesia, P.O. Box MP 167, Mount Pleasant, Salisbury, Rhodesia. Overseas applicants should send an additional copy to Association of Commonwealth Universities (Apts.), 36 Gordon Square, London WC1H 0PF. Further particulars may be obtained from either of the above.

Closing date for receipt of applications: **September 15, 1978**, but will be accepted for a further 14 days.

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 233 4143) or their nearest British Consular Office.

7000(A)

UNIVERSITY OF KENT AT CANTERBURY EXPERIMENTAL OFFICER/ SENIOR EXPERIMENTAL OFFICER IN ELECTRONICS

Applications are invited for the post of Experimental Officer/Senior Experimental Officer to operate and develop solid state equipment, clean air suites and a 2 Mv Van der Graaf generator in the Electronics Laboratories. Applicants should have a degree in Electronics or a related subject but other candidates with relevant experience will also be considered. Salary scales: £3,189 to £5,282 and £3,660 to £6,178.

Application forms and particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7N1 to whom completed applications should be returned by September 29, 1978. Please quote ref: A31/78. 7034(A)

THAMES POLYTECHNIC SCHOOL OF BIOLOGICAL SCIENCES RESEARCH IN MICROBIAL GENETICS

Applications are invited from good honours graduates for the post of M.R.C. Research Assistant to Dr A. Vivian to work on the genetics of *Acinetobacter calcoaceticus*. This is a three year project funded by the Medical Research Council and the appointment is for a limited period (maximum three years). The successful applicant will be registered with the C.N.A.A. for a higher degree.

Salary: £3,015 to £3,189 inclusive. Further particulars and form of application may be obtained from the Staffing Officer, Thames Polytechnic, Wellington Street, London SE18 6PF, to whom completed applications should be returned by September 19, 1978.

6080(A)

SCOTTISH HORTICULTURAL RESEARCH INSTITUTE INVERGOWIE, DUNDEE DD2 5DA SCIENTIFIC OFFICER

A Scientific Officer is required in the Zoology Section to study the nature of tolerance in potatoes to potato cyst nematodes. Applications are invited from persons with training in plant physiology, plant pathology or nematology.

The project is financed by the Potato Marketing Board for a period of three years and the successful candidate may have the opportunity to work for a higher degree. The minimum qualification is an Upper Second Class Honours degree.

Salary: £3,294 to £3,587. Additional increments may be awarded for relevant post-qualifying experience.

Further particulars may be obtained from the Secretary of the Institute with whom applications naming two referees and stating age, education and experience should be lodged by September 30, 1978.

7044(A)

UNIVERSITY OF EDINBURGH RESEARCH FELLOW ON THE CELL CYCLE

Applications are invited for the post of Postdoctoral Fellow (Research Fellow) to work in the Department of Zoology under the direction of Professor J. M. Mitchison. The research would be on the biochemistry and genetics of the yeast cell cycle, and applicants will be favoured if they have had experience in either genetic or biochemistry of yeast.

This post is financed by a Science Research Council grant and the salary would be on the Research Range 1A starting at not more than point 3.

Applications should be sent to Professor J. M. Mitchison, Department of Zoology, West Mains Road, Edinburgh EH9 3JT, giving the names of two referees. The closing date is: September 30, 1978. Please quote reference 5021.

7056(A)

AGRICULTURAL RESEARCH COUNCIL INSTITUTE OF ANIMAL PHYSIOLOGY

Babraham, Cambridge CB2 4AT

A vacancy exists for a Scientific Officer with experience in biochemical techniques to join a small research group studying gene expression in reproductive tissues particularly with respect to the hormonal regulation of specific proteins. Research techniques will include nucleic acid hybridization and cell-free protein synthesis. Qualifications required are: Pass degree, H.N.C. or equivalent. Salary in S.O. scale (£2,839 to £4,415 p.a.) according to qualifications and experience. Non-contributory superannuation scheme. Applications to the Secretary of the Institute by September 14, 1978. Quote Ref. ARS 9.

7027(A)

FRESHWATER BIOLOGICAL ASSOCIATION WINDERMERE LABORATORY ASSISTANT LIBRARIAN/ INFORMATION SCIENTIST

Applications are invited from graduates in information science, or science graduates with a qualification in Librarianship, for the above post which is to strengthen the Association's library and information services. The post will be graded as Assistant Librarian (£3,113 to £3,579 per annum), and conditions of service will be generally similar to those of the Civil Service. Full details and application form may be obtained from the Secretary, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 0LP. Closing date September 30, 1978.

7025(A)

**BIRKBECK AND IMPERIAL
COLLEGES****(University of London)
RESEARCH OFFICER IN
MOLECULAR COMPUTER
GRAPHICS**

Applications are invited for a three-year post funded by the Science Research Council to work jointly with Professor D. M. Blow and Professor T. L. Blundell in the development of interactive computer graphics for structure analysis of biological systems including proteins, drug molecules and macromolecule-water interactions.

The hardware includes an Evans and Sutherland Picture System II driven by a PDP11/60 computer linked to an IBM70/165 mainframe computer.

Applicants should have postgraduate experience in computing, structure analysis or related fields.

Salary according to qualifications and experience, within Grade 1A; entry point not exceeding £4,408 plus London Weighting £450.

Applications to Professor T. L. Blundell, Department of Crystallography, Birkbeck College, Malet Street, London WC1E 7HX, telephone no. 01-580 6622. 7002(A)

**CENTRAL
HAEMATOLOGY
LABORATORY**

Inselspital, Berne, Switzerland

Applications are invited for the post of

**RESEARCH BIOCHEMIST
(POSTDOCTORAL
RESEARCH FELLOW)**

for a period of two years from November 1, 1978. The project is concerned with the relationship between structure and function of fibrinogen or factor VIII. Candidates should have experience in biochemistry of biological macromolecules. Salary will be approximately SFR. 3,000 per month before taxes.

Applications, including a curriculum vitae and the names of two referees, should be sent to Dr M. Furlan, Hämatologisches Zentrallabor, Inselspital, CH-3010 Bern. 7050(A)

**ROCHE
RESEARCH INSTITUTE OF
MARINE PHARMACOLOGY**

invites applications for the position of

SENIOR MARINE BIOLOGIST

The Roche Research Institute of Marine Pharmacology has been established at Dee Why, N.S.W., Australia to investigate the chemistry, chemotherapy and pharmacology of natural products from marine organisms.

Applicants for the position of Senior Marine Biologist should hold a post-graduate university degree, or its equivalent, with a major specialisation in marine zoology, botany or biology, and possess an extensive knowledge of, and interest in, marine organisms and their habitat. Qualification and experience in scuba diving would be an advantage.

This appointment will be the senior position of the marine biology staff consisting of three graduate scientists. The senior marine biologist will organise and co-ordinate the collecting activities for the Roche research institute and must be able to collaborate with research scientists within the institute and with collectors in the field. The senior marine biologist may be required to spend extended periods away from Sydney, establishing areas and facilities for collection.

An attractive salary will be negotiated according to qualifications and experience. Generous superannuation benefits are available.

Applications, together with supporting statements from two referees, should be forwarded to the Director, Roche Research Institute of Marine Pharmacology, P.O. Box 255, Dee Why, NSW, 2099, by Friday, October 16, 1978. 6089(A)

R & D Management in Agrochemicals

South East England from £10,000 plus car

A Research Manager and a Development Manager are required to join the senior team directing the substantial R & D effort of one of the UK's foremost agrochemical businesses. A division of a well-known British company and part of one of the world's ten largest chemical groups, the business has a fine reputation for research-led product innovation and an impressive record of export achievement. The Division has its own excellent research facilities located in a pleasant part of South East England and has ready access to the considerable resources of the parent organisation. Both managers will report to the Research & Development Manager. There are good prospects of career progression within the company.

Research Manager

This post carries responsibility for identifying viable research topics and for the planning, direction and co-ordination of departments comprising multi-disciplinary teams. Scientists in each team are engaged in the chemical synthesis and biological testing of novel chemicals in the search for new herbicides, insecticides, plant growth regulators and fungicides which show sufficient promise to progress to the development stage. The Research Manager will also be responsible for liaison with other departments within the Division and the

maintenance of good relationships with researchers both within the parent organisation and at universities and other research establishments. Probably aged 35 or over, candidates, men or women, must have spent a minimum of 8 years in agrochemical or pharmaceutical research and have a record of successful discovery. Experience of directing the work of other scientists is essential as is the possession of a higher degree in chemistry, biochemistry or biology.

PA Personnel Services Ref: TE54/6539/N

Development Manager

Through a team of departmental heads the Development Manager will control the work of a large group of scientists and support staff. These are engaged in the field testing of active compounds to determine their efficacy and crop safety, and the appropriate method of application. Field experiments and trials are organised both in the UK and overseas and the Development Manager will be involved in regular overseas visits. Close

liaison with other senior managers in the Division is entailed. Candidates, men or women, probably aged 35 or more, must have spent at least 8 years in the agrochemicals industry and have experience in directing the work of other scientists. A degree in agriculture, chemistry, biochemistry or biology would be the minimum qualification.

PA Personnel Services Ref: TE54/6540/N

An ability to read French and German would be useful. The salary for each appointment is negotiable within a range starting at £10,000 per annum. A car is provided and the usual large company benefits apply including assistance in relocation where necessary.

Initial interviews are conducted by PA Consultants. No details are divulged to clients without prior permission. Please send brief career details or write for an application form, quoting the reference number on both your letter and envelope, and advise us if you have recently made any other applications to PA Personnel Services.

PA Personnel Services

Hyde Park House, 60a Knightsbridge, London SW1X 7LE. Tel: 01-235 6060 Telex: 27874



A member of PA International

6060(A)

**THE UNIVERSITY OF
MANCHESTER
RESEARCH ASSISTANT
IN PSYCHOLOGY**

Applications are invited for this post from graduates in Psychology. The work will be concerned with cognitive development in young children. The appointment will initially be for a period of two years. Salary range p.a.: £3,660 to £4,365. Superannuation. Applications, naming two referees, to M. S. Halliday, Department of Psychology, The University, Manchester M13 9PL, by September 29, 7004(A)

**UNIVERSITY
OF CAMBRIDGE
DEPARTMENT OF PATHOLOGY
TECHNICIAN**

required from November 1 for work on project involving the cultivation of trypanosomes for genetic and immunochemical studies. Qualifications HNC or equivalent. Experience of tissue culture techniques and practical biochemistry advantageous. Post funded from outside sources until September 30, 1981. Salary range £2,959 to £3,409. Applications in writing to The Superintendent, Department of Pathology, Tennis Court Road, Cambridge CB2 1QP. 6064(A)

**UNIVERSITY OF GLASGOW
DEPARTMENT OF
VETERINARY PATHOLOGY**

Applications are invited from pre or postdoctoral graduates with immunological training to work on a project concerning the immunoregulation of IgE. The position, which is on salary range 1A for research staff, is funded by the Wellcome Trust and is available immediately.

Applications and enquiries to Dr E. Jarrett, University Veterinary Hospital, Bearsden, Glasgow G61 1QH. Telephone: 041-942 2301 extension 54. In reply please quote Ref. No. 4235.



Oxford
University Press

Oxford English Dictionary Scientific Research Assistant

The Oxford University Press has a vacancy in Oxford for a full-time research assistant for *A Supplement to the Oxford English Dictionary*, now in preparation. Volume I, A-G, was published in 1972 and Volume II, H-N, in 1976. The successful applicant will undertake research in Oxford libraries into the history and usage of specific scientific terms.

Candidates should have an honours degree in a scientific subject and an interest in words and the use of library resources. A reading knowledge of German is desirable.

Salary according to age and experience on the scale £2771 - £4233, with participation in the Oxford University Press Superannuation Scheme.

Applications should be made in handwriting to L. R. Swanzy, Personnel Department, Oxford University Press, Walton Street, Oxford OX2 8DP by 30 September 1978, with the names and addresses of two referees.

7041(A)

EUROPEAN MOLECULAR BIOLOGY LABORATORY

MOLECULAR COMPUTER GRAPHICS

Applications are invited for a post in the Instrumentation Division to develop interactive computer graphics software for macromolecular structure analysis and other biological problems. Applicants must have interest and experience in development, maintenance and documentation of a library of advanced computer programs and should also be familiar with crystallographic computing, theoretical chemistry (for example molecular dynamics), or interactive computer graphics.

Hardware includes an Evans and Sutherland Picture System II driven by a 128 K-word minicomputer. Access to a remote DEC 1090 and to an Amdahl 470/IBM 360-91 system is also available. Present software includes an extension of the Diamond interactive molecular graphics system.

Salary, according to qualifications and experience, within range DM 3400 and DM 4000 monthly, after tax. Removal costs and, where appropriate, expatriation and other allowances, will be paid.

Applications, quoting reference No. 78/28, and including a curriculum vitae, brief statement of research interest and the names of referees should be sent to:

Sir John Kendrew
Director General
EMBL
Postfach 10.2209
D - 6900 Heidelberg
Germany

6095(A)

QUEEN ELIZABETH COLLEGE Kensington

(University of London)
DEPARTMENT OF FOOD
SCIENCE AND NUTRITION

RESEARCH STUDENTSHIP

Applications are invited for a Research Studentship, sponsored by a major food manufacturer, to work with Dr Stuart Thorne on a feasibility study of a novel food preservation process. Applicants should have a good, relevant honours degree, some mathematical ability and an interest in unsteady state heat and mass transfer. The successful candidate will be expected to spend some time at the sponsor's premises.

The studentship is tenable for two years in the first instance and may be renewed for a third year. Support will be at the same rate as the student would receive from a Research Council, and travelling and subsistence allowances whilst working at the sponsor's premises will be given.

Applications, including a brief curriculum vitae and the names and addresses of two academic referees should be sent to Dr S. N. Thorne, Queen Elizabeth College, Campden Hill Road, Kensington, London W8 7AH, by September 30, who may also be contacted informally (01-937 5411 extn 238).

7054(A)

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF GEOLOGICAL SCIENCES LECTURER

IN HYDROGEOLOGY

Applications are invited for the above post, the duties of which will principally include the teaching of Hydrogeology at M.Sc. Course level and the supervision of Course project work. The successful applicant will be expected to pursue research in the field of hydrology.

Applicants should have a good honours degree. A postgraduate qualification in Hydrogeology and practical experience would be advantageous.

Salary according to age and experience, in the range £3,660 to £7,308 (to be reviewed from October 1) plus superannuation.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, PO Box 363, Birmingham B15 2TT, to whom applications (3 copies) including curriculum vitae and the names of three referees should be sent by Friday, October 20, 1978. Please quote reference NG6

6073(A)

DIRECTOR of the proposed CRUMP INSTITUTE OF MEDICAL ENGINEERING AT U.C.L.A.

Applications are invited for consideration by the Search Committee for the Director of the proposed "Crump Institute for Medical Engineering" at U.C.L.A.

A new building is being planned, a Chair has been endowed for the Director, and private and University core support has been pledged. This position represents a unique opportunity to contribute to the development of the evolving field of "medical engineering," and to establish a major Medical Engineering Programme at U.C.L.A.

Qualifications: A person of exceptional ability, accomplishments, and international stature is sought whose work and interests bear on the interface between engineering and medical sciences. Demonstrated scientific productivity, leadership and administrative abilities are desirable.

Responsibilities: To administer and direct the proposed Institute.

Salary: Commensurate with qualifications and experience.

Equal Opportunity: The University of California is an equal opportunity employer.

Send resume to Dr Frederick Allen, Chairman, Search Committee, School of Engineering and Applied Sciences, 7714 Boelter Hall, U.C.L.A., Los Angeles, CA 90024.

6098(A)

UNIVERSITY OF WARWICK POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a one year Postdoctoral Research Fellowship in the Department of Chemistry and Molecular Sciences to work on the photochemistry of metal complexes in the solid state with Drs N. W. Alcock and T. J. Kemp. The project will involve synthesis, photochemistry and X-ray diffraction and would be suitable for someone with expertise in one of these fields (particularly the first) wishing to extend their range of skills. The post is available immediately. Initial salary will be on one of the first three points of the Research Range 1A scale: £3,660 (£4,130) to £6,178 p.a. (under review).

Applications (including a detailed curriculum vitae and the names of two referees) to the Academic Registrar, University of Warwick, Coventry CV4 7AL by October 6, 1978. Please quote Ref No. 3/A/78. 6072(A)

HEINRICH PETTE- INSTITUT

für Experimentelle Virologie
und Immunologie an der
UNIVERSITÄT HAMBURG

Applications are invited for the position of

HEAD OF THE DIVISION OF IMMUNOLOGY

The Institute consists of six divisions. It is a foundation concerned with research on the biology of human viruses, the pathogenesis of virus diseases, and defence mechanisms of the host and related problems.

We are looking for a scientist whose main interest lies in the field of cell biology and/or virology.

Curriculum vitae, list of publications, and further documents should be sent to the Board of Directors of the Heinrich Pette-Institut, Martinstrasse 52, D-2000 Hamburg 20, Federal Republic of Germany, by November 1, 1978. 6092(A)

MASSEY UNIVERSITY Palmerston North, New Zealand LECTURER/SENIOR LECTURER IN PIG HUSBANDRY AND DIRECTOR OF PIG RESEARCH CENTRE

Applications are invited for the above mentioned position. The successful applicant will become a member of the Dairy Husbandry Department responsible to the Head of that Department, for teaching and to the Vice-Chancellor, for the Pig Research Centre.

At present pig production courses involve about one hundred hours of undergraduate teaching annually plus postgraduate student teaching and supervision.

The Pig Research Centre has a staff of one Research Officer and three permanent stockmen. The Director reports to the University Farms Committee, a subcommittee which is made up of University Council Appointees and New Zealand Pork Industry Council members. The Centre is a University Facility, funded jointly by the Pork Industry Council, the Ministry of Agriculture and Fisheries, and the University.

Salary: Senior Lecturer NZ\$13,424 to NZ\$15,411; Lecturer NZ\$10,585 to NZ\$12,969.

Further details of the position together with the conditions of appointment may be obtained from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or the Registrar of the University. Applications close on September 29, 1978. 7008(A)

UNIVERSITY OF
MELBOURNE
LECTURESHIP IN THE
SCHOOL OF PHYSICS

Applications are invited for a continuing Lectureship position in the School of Physics. The current research interests of the School are high energy physics, diffraction physics, low energy nuclear physics and theoretical physics.

The successful applicant will be required to teach at an undergraduate level by lecturing or supervising laboratory classes, to supervise research students and to conduct research in one of the above areas of physics.

QUALIFICATIONS Ph.D. with postdoctoral experience.

SALARY \$A15,179 to \$A19,940 per annum.

The position can be taken up on January 16, 1979, or as soon as possible thereafter.

Further information, including details of application procedure and conditions of appointment, is available from the Registrar, University of Melbourne, Parkville, Victoria, Australia 3052, or from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. Applications close October 31, 1978.

7064(A)

CHELSEA COLLEGE
University of London

A POSTDOCTORAL RESEARCH ASSISTANT is required to join a group working on biological membranes. Particular experience in the biophysical aspects of the subject would be an advantage. The post is supported by the Muscular Dystrophy Association and the appointment will be for one year in the first instance to commence as soon as possible. Salary in the range £3,660 to £4,130 per annum plus £450 London Allowance.

Application forms and further details from the Personnel Officer, Chelsea College, Friesse Greene House, Chelsea Manor Street, London SW3 3TW. Closing date September 25, 1978.

7042(A)

DEAKIN UNIVERSITY
Geelong, Australia
SCHOOL OF SCIENCES
LECTURER IN PHYSIOLOGY
Ref: 78/1/12

Applications are invited from suitably qualified people for the above-mentioned position.

In 1979 the University will have about 4,000 students, of which about half will be enrolled in off campus courses. The main campus of the University is on an 80 hectare site at Warrn Ponds, 8 kilometres south west of Geelong.

A fixed term appointment is normally for period of three years. Although an appointment may be extended for a further three years it cannot be translated into a permanent appointment.

Current Salary Range \$A15,179 to \$A19,940 per annum.

The Division of Biological and Health Sciences offers undergraduate major programs in biology, biochemistry, nutrition and food chemistry, a graduate diploma of dietetics, and participates in the teaching of a graduate diploma of industrial hygiene. Present research interests of the Division are in the areas of toxicology, endocrinology and nutrition.

The appointee will assist with the lecturing in undergraduate physiology and biochemistry courses, with a major emphasis on physiology. Applicants should possess a doctorate in an appropriate discipline and have some tertiary teaching experience. The appointment is offered on a fixed term (three years) basis to commence on January 1, 1979.

Applications (quoting reference number) should include a full curriculum vitae and the names and addresses of three academic referees, and should be sent to reach the Staff Officer at P.O. Box 125, Belmont, 3216, Victoria, Australia by October 1, 1978. Applicants resident in North America, Europe or Africa should also send a copy of their applications to the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF, from whom further details about the position can be obtained.

7064(A)

AUSTRALIAN INSTITUTE OF MARINE SCIENCE

Applications are invited for the following position in research at the Australian Institute of Marine Science (A.I.M.S.), which is situated on a 190 hectare site at Cape Ferguson near Townsville, North Queensland, and projects development into one of the foremost institutions in the world in the field of tropical marine science. Facilities include a 12.8 million dollar headquarters and comprehensively equipped laboratory complex, library, lecture theatre, controlled environment rooms and aquaria, accommodation for visiting scientists, and various field and technical support facilities such as a deepwater boat harbour, recompression chamber, and maritime services building.

Initial research at A.I.M.S. has been concentrated in three main areas:

- (I) Tropical marine food webs
- (II) The Great Barrier Reef and its Reef-building organisms
- (III) Tropical marine pollution.

The existing fleet of small vessels will shortly be augmented substantially, with the delivery of a 26m research vessel built specifically for A.I.M.S.

Further major expansion is planned, and will assure challenging research growth in tropical marine science.

BIOLOGICAL OCEANOGRAPHER

Duties: To plan and execute field/laboratory programmes devoted to a fuller understanding of the structure, function and dynamics of pelagic food webs, especially within coastal and oceanic waters of the tropics. Consideration of likely interactions with shallow benthic and land-sea interface systems are viewed as potentially significant.

Qualifications: A Ph.D. degree or equivalent qualifications in the field of biological oceanography with evidence of substantial contribution in research and experience in the management of programmes at sea and ashore. A specific area of interest is not pre-requisite, however, the individual selected must be equipped to view his or her responsibilities with perspective within the broad limits of the discipline.

Appointment will be made within the Senior Research Scientist range (\$A18,819-\$A21,545), depending on qualifications and experience. Appointment at a higher level may be considered in exceptional circumstances.

It is envisaged that the successful applicant will be required to take up appointment on April 1, 1979.

Further information on A.I.M.S. and general employment conditions will be supplied on request.

Application must include a full c.v., plus the names and addresses of three professional referees, and should be sent to:

The Secretary, Australian Institute of Marine Science, P.M.B. No. 3, TOWNSVILLE, M.S.O. 4810

Closing Date for applications is October 20, 1978.

7017(A)

QUEEN'S MEDICAL CENTRE
UNIVERSITY OF
NOTTINGHAM
DEPARTMENT OF
BIOCHEMISTRY

A RESEARCH TECHNICIAN is required to assist on a research funded project on the biochemical mechanism and control of the biosynthesis of fungal wall polysaccharides and glycoproteins. The appointment is available from October 1, 1978 and is available for one year in the first instance. The post is on the Whitley Scale and commences at a salary of £3,261 rising to a maximum of £4,680 depending on age, qualifications and experience. The successful applicant is expected to have experience and/or interest in standard microbiological and biomedical techniques.

Application forms from Mr F. C. Treeby, Chief Technical Adviser, Medical School, Queen's Medical Centre, Nottingham NG7 2UH

6082(A)

NATAL MUSEUM
Pietermaritzburg, South Africa
VACANCY FOR A
DIPTERIST

Applications are invited for a vacancy in the Department of Entomology at the Natal Museum. The appointee will be required to take charge of the Department and undertake taxonomic research on Diptera. A person interested in one of the families Tachinidae, Cecidomyiidae, Dolichopodidae or Phoridae, is particularly sought, but applicants with other interests will also be considered. The minimum academic requirement is an Honours degree. Full details of the post and application forms can be obtained from the Director at 237 Loop Street, Pietermaritzburg 3201, South Africa.

7033(A)

PORTSMOUTH
POLYTECHNIC
DEPARTMENT OF GEOLOGY
LECTURER II/
SENIOR LECTURER

Structural Geologist preferably with experience and interest in Engineering Geology to teach B.Sc. (Honours) courses and undertake research. From January 1, 1979.

Salary scale: £4,101 to £6,051 (efficiency bar) to £7,572 per annum.

Further particulars and application forms from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth PO1 2QQ to whom completed applications should be returned by September 19, 1978. Please quote ref: X12 6083(A)

EUROPEAN MOLECULAR BIOLOGY LABORATORY

X-RAY CRYSTALLOGRAPHER

Applications are invited for a post in the Biological Structures Division to study macromolecular structure using X-ray diffraction. Applicants should have experience in crystallographic computing, crystallography of macromolecules, or X-ray diffraction analysis of semicrystalline materials such as fibres.

The research programme includes studies of structures involved in the mechanism and control of DNA replication, in membrane function, and in virus assembly, especially using the filamentous bacterial virus system as a model.

Salary, according to qualifications and experience, within range DM 3400 and DM 4000 monthly, after tax. Removal costs and, where appropriate, expatriation and other allowances, will be paid.

Applications, quoting reference No. 78/28, and including a curriculum vitae, brief statement of research interests and the names of referees should be sent to:

Sir John Kendrew
Director General
EMBL
Postfach 10.2209
D — 6900 Heidelberg
Germany

6096(A)

LADY DAVIS FELLOWSHIP TRUST

P.O. Box 1255, Jerusalem, Israel

FELLOWSHIPS FOR STUDY AND/OR RESEARCH AT GRADUATE OR POSTGRADUATE LEVELS AND VISITING PROFESSORSHIPS

in 1979–80

at the Hebrew University of Jerusalem and at the Technion—
Israel Institute of Technology, Haifa

GRADUATE AND POSTDOCTORAL FELLOWSHIPS

Eligibility: Lady Davis Fellows will be selected on the basis of demonstrated excellence in their studies, promise of distinction in their chosen fields of specialisation and qualities of mind, intellect and character.

Postdoctoral candidates shall have completed their doctoral dissertation (before arrival in Israel).

The Graduate and Postdoctoral Fellowships are tenable for a period of one year. They may be renewed for a second year and in special circumstances extended to a third year.

The grant is intended to defray the cost of the Fellow's travel and tuition fees and to meet reasonable living expenses.

Application: Graduate candidates may apply during their senior academic year or after they have undertaken study in a graduate school. Postdoctoral candidates may apply at an early stage of their professional career (not later than three years after completion of their doctoral dissertation).

Graduate and Postdoctoral candidates (including Israelis abroad) may obtain application forms from the Lady Davis Fellowship Trust at the above address, after they indicate the category of Fellowship for which they are eligible.

Completed applications must reach the office of the Trust not later than January 1, 1979.

VISITING PROFESSORSHIPS

These Fellowships are intended for candidates with the rank of Full or Associate Professor at their own institution. They are tenable for periods from one trimester (or semester) to a year.

Application: Forms may be obtained from the Lady Davis Fellowship Trust at the above address. Completed application forms for the Hebrew University must reach the office of the Trust by November 30, 1978. Those for the Technion by December 31, 1978.

7045(A)

BASIC GRADE BIOCHEMIST FACULTY OF MEDICINE DIAGNOSTIC RESEARCH UNIT SOUTHAMPTON GENERAL HOSPITAL

A biochemistry graduate is required to assist in the development of diagnostic methods for use in hospital biochemical laboratories. Appointment one year in the first instance. Salary: £3,486 to £4,275 per annum according to relevant experience. Application forms and job description from District Personnel Department, Tremona Court, 30-32 Tremona Road, Southampton. Telephone: 777222, Ext. 3556 or 3018. Further information from Dr F. G. Bull, Telephone: 777222, Ext. 3910. Closing date: September 21, 1978.

6085(A)

UNIVERSITY OF SURREY DEPARTMENT OF BIOCHEMISTRY LECTURER IN BIOCHEMISTRY

Applications are invited from suitably qualified graduates for a Lectureship in Biochemistry in the Department of Biochemistry. Preference will be given to applicants with postgraduate experience and to those with an interest in pharmacological biochemistry. Industrial experience would also be considered advantageous.

Salary will be within the range £3,660 to £7,308 per annum according to qualifications and experience. Superannuation will be under U.S.S. conditions.

Further particulars about the post may be obtained from the Academic Registrar (LFG), University of Surrey, Guildford, Surrey GU2 5XH or Tel. Guildford 71281, ext. 452. Applications from men and women in the form of a curriculum vitae, together with the names and addresses of two referees, should be sent to the above by September 29, 1978.

6070(A)

NEUROSCIENCES FACULTY

Faculty positions available in the Neurosciences Program, Maryland Psychiatric Research Center, affiliated with the University of Maryland School of Medicine. One position is open to a

SCIENTIST

with rigorous postgraduate training in neuroendocrinology, basic expertise in hormone assay procedures and the capability to study functional aspects of hormones/polypeptides in a multidisciplinary clinical research centre. The second position is for a SCIENTIST with rigorous postgraduate training in a neurobiology specialty with preference given to strength in biochemistry and/or genetics plus experience in cell or organ culture techniques.

Submit curriculum vitae, summary of research interest and two letters of reference by November 15, 1978, to: Neurosciences Search Committee, Maryland Psychiatric Research Center, Box 3235, Baltimore, Md. 21228. An Equal Opportunity Employer.

7060(A)

UNIVERSITY OF WARWICK RESEARCH ASSOCIATE

in the

DEPARTMENT OF CHEMISTRY AND MOLECULAR SCIENCES

Applications are invited for the post of RESEARCH ASSOCIATE with Dr B. E. P. Swoboda to study the detailed mode of action of the anti-tumour agent, cyclophosphamide on normal and cancer cells. Some experience with cell culture, organic syntheses and/or radioisotope techniques would be advantageous and the applicant should have a good degree in Biochemistry or a related subject.

The appointment, which is supported by the Cancer Research Campaign, is initially for one year, with the possibility of extension to three years. Maximum starting salary will be £3,660 per annum on Research Range 1B, plus superannuation. Applications (two copies) giving qualifications and experience and naming two referees should be sent to the Academic Registrar, University of Warwick, Coventry CV4 7AL, as soon as possible, quoting Ref. No. 4/2A/78.

6084(A)

THE LONDON HOSPITAL MEDICAL COLLEGE (University of London) DEPARTMENT OF BIOCHEMISTRY RESEARCH ASSISTANT

Applications are invited for the post of Research Assistant in the Department of Biochemistry. The post will be tenable for a period of two years with effect from October 1978.

The project with which the successful applicant will be associated concerns the immunochemical aspects of human lysosomal enzymes in relation to two lysosomal storage disorders, Tay-Sachs disease and Gaucher's disease. The project is funded by the Mental Health Foundation.

Candidates should have a B.Sc. degree in biochemistry as well as appropriate practical experience and an interest in the field of human lysosomal enzymes. It is unlikely that there will be the opportunity to study for a higher degree.

Salary: The basic salary will be £3,384 plus £450 London Allowance per annum together with superannuation under the U.S.S.

Apply in the first instance with a curriculum vitae and the names and addresses of two academic referees to: Dr Mark Carroll, Department of Biochemistry, The London Hospital Medical College, Turner Street, LONDON E1 2AD.

7003(A)

UNIVERSITY OF SURREY DEPARTMENT OF BIOCHEMISTRY READER IN NUTRITION

Applications are invited for the position of Reader in Nutrition in the Department of Biochemistry. The person appointed will contribute to the undergraduate and postgraduate teaching within the Department and be responsible for the initiation and supervision of research.

Applicants must be graduate with a higher degree and a good research record. Preference will be given to those with an interest and experience in clinical nutrition. A medical qualification would be an additional recommendation.

Salary will be within the range £7,074 to £8,730 per annum according to qualifications and experience. Superannuation will be under U.S.S. conditions.

Further particulars about the post may be obtained from the Academic Registrar (LFG), University of Surrey, Guildford, Surrey GU2 5XH or Tel. 71281, ext. 452. Applications from men and women in the form of a curriculum vitae, together with names and addresses of three referees, should be sent to the above by September 29, 1978.

6069(A)

UNIVERSITY OF ASTON IN BIRMINGHAM DEPARTMENT OF GEOLOGICAL SCIENCES

Applications are invited for two new posts as

LECTURER IN GEOLOGY

Candidate for the first post should have active research interests in the field of igneous petrology, preferably involving rocks of Mesozoic or later age.

The second post is open to active research workers in any field of geology. It is unlikely, however, that the post will be given to a specialist mineralogist or palaeontologist.

Possession of a Ph.D. degree is a condition of appointment, but young research workers who anticipate the award of a doctorate by November 1978 may apply.

Commencing salary will be within the range £3,883 to £5,842 p.a. on a scale rising by annual increments to £7,754 per annum. (Under review).

Preliminary information about these posts may be obtained from Professor D. D. Hawkes (Tel. 021 359 3611 Ext. 568) at the following address. Application forms and further details may be obtained from the Staff Officer (Ref. 290/6) University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (Tel. Ext. 201) to whom applications should be forwarded not later than Friday, September 29, 1978.

6079(A)

M.R.C. CLINICAL RESEARCH CENTRE
(Northwick Park Hospital)
Watford Road, Harrow,
Middx. HA1 3UJ

**APPOINTMENT OF
HEAD OF DIVISION OF COMPUTING AND STATISTICS**

The Medical Research Council invites applications for the post of head of the division of computing and statistics at the Clinical Research Centre, Harrow, Middx.

The person appointed will be required to direct the division's research programme, to maintain a computing and statistical service for the Clinical Research Centre, and also to maintain a close association with the Northwick Park Hospital Computing Service. The future research programme will be a matter for discussion between the new head of the division and the director of the centre, taking into account the particular interests of the selected candidate and the needs of the centre.

Candidates should be of a high scientific standing and with proven ability in managing a research team.

The salary will be within the range for the council's special appointments grade which is equivalent to the universities' professorial range.

Further information about the appointment may be obtained from the Director, Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ. Ref. 109/1/4079. The final date for the receipt of application is November 3, 1978. 7061(A)

**UNIVERSITY OF
ST ANDREWS**
DEPARTMENT OF ANATOMY
AND EXPERIMENTAL
PATHOLOGY

Applications are invited for the recently allocated post of LECTURER in the Department of Anatomy and Experimental Pathology. The successful candidate will be expected to contribute to the courses that are provided for pre-clinical students and for science students. Excellent facilities for research are available. The department may be visited by arrangement with Mrs Norton (telephone Andrews 2411).

Salary at appropriate point on scale 3,660 to £7,308 (under review), starting salary probably not above £5,282. S.S.U./U.S.S.

Applications (two copies preferably, typed) with the names of three referees should be sent by September 2, 1978 to the Establishments Officer, The University, College Gate, St Andrews, Fife, from whom further particulars may be obtained. 7013(A)

**THE WEST OF SCOTLAND
AGRICULTURAL COLLEGE**
SENIOR LECTURER/
ADVISED IN POULTRY
HUSBANDRY

Applications for the post are invited from holders of a suitable degree or a nationally recognised diploma with considerable relevant experience. The duties will include formal teaching of 11-time students, advisory work for the poultry industry and related research/development activity.

The salary will be on Grade II, £4,477 to £7,651 per annum. Further particulars and application forms may be obtained from the Secretary, The West of Scotland Agricultural College, Ochmercurie, Ayr KA6 5HW, with whom applications should be lodged at later than October 20, 1978. 7036(A)

**THE UNIVERSITY OF
SHEFFIELD**
DEPARTMENT OF METALLURGY
POSTDOCTORAL
RESEARCH ASSISTANT
(S.R.C.)

Physicist, chemist, chemical engineer, metallurgist or materials scientist (man or woman) required for light-scattering studies on aerosol condensation using visible and infrared laser radiation. Initial salary in range £3,660 to £4,365 according to qualifications and experience, with superannuation. Tenable 2 years. Curriculum vitae with names and addresses of two referees to Dr E. R. Buckle, Department of Metallurgy, the University, Sheffield S1 3JD. Quote ref. R194/G-7015(A)

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Details of posts and application forms from Administrative Officer, Faculty of Natural and Life Sciences, Brighton Polytechnic, Moulsecoomb, BRIGHTON BN2 4GJ, to whom completed forms should be returned by September 18, 1978. 7027(A)

**THE LADY DAVIS
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**UNIVERSITY OF
NOTTINGHAM**
GENETICS RESEARCH UNIT
RESEARCH TECHNICIAN
GRADE 3

This post involves helping in a research project, financed by the Medical Research Council, on the genetic control of enzyme synthesis in the fruit fly. Minimum educational requirements: O.N.C., O.N.D. or equivalent. Credit will be given for H.N.C., H.N.D. or degree. Experience in biochemical techniques is desirable. The appointment is for two years at a salary in the range £2,638 to £3,060, according to age and experience.

Applications, including a curriculum vitae, giving details of qualifications and experience and the names of two referees should be sent to Professor Bryan Clarke, Genetics Research Unit, Queen's Medical Centre, Clifton Boulevard, Nottingham NG7 2UH before October 15, 1978. 7020(A)

**THE UNIVERSITY
OF MANCHESTER**
GEORGE HARRISON
CHAIR OF BOTANY

Applications are invited for the George Harrison Chair of Botany which will fall vacant on October 1, 1979 on the retirement of Professor D. H. Valentine. The other Chair in Botany is the Barker Chair of Cryptogamic Botany which is held by Professor John Colhoun. Particulars of the Appointment may be obtained from the Registrar.

Applications (2 copies, 1 suitable for photocopying), giving full details of qualifications, experience, research etc., and the names and addresses of three persons to whom reference may be made, should be sent to the Registrar, The University, Manchester M13 9PL by October 18, 1978. 6067(A)

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Bush Boake Allen Ltd., a division of Albright & Wilson, have a vacancy for a Biochemist/Microbiologist to work in their microbiological research laboratory, based at Walthamstow, London E.17.

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stating age, qualifications and
experience. 7032(A)

Bush Boake Allen

UNIVERSITY COLLEGE GALWAY

JUNIOR LECTURESHIP IN GEOLOGY

Applications are invited for the above post. Salary scale £4,757 x (10) — £6,575. Closing date for receipt of applications 6 OCTOBER 1978. Further information may be obtained from the Registrar. 7014(A)

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■ Application forms (for return by 28 September 1978) from Miss M Cole, Centre for Overseas Pest Research, College House, Wrights Lane, London W8 5SJ.

6059(A)

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THE CALIFORNIA INSTITUTE OF TECHNOLOGY

invites applications for a
JUNIOR FACULTY
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The major research interests of candidates should be in observational optical or infrared astronomy. The position is an assistant professorship; however, in exceptional circumstances the successful candidate may be offered an associate professorship.

Members of the Caltech Astronomy Faculty whose major research activity is in optical or infrared astronomy are normally appointed as Staff Members of the Hale Observatories subject to the approval of the California Institute and of the Carnegie Institution of Washington.

Applications should include (1) a description of educational background; (2) a résumé of research and teaching experience; (3) a list of all publications; (4) copies of all manuscripts submitted for publication but not yet published; (5) a statement of current research interests and future research plans. The applicant should ask three scientists who know the applicant personally to send letters of evaluation directly to Professor W. L. W. Sargent, Executive Officer for Astronomy, California Institute of Technology, Pasadena, CA 91125. Completed applications and letters of evaluation should be received by November 1, 1978; appointment will be made as soon thereafter as circumstances permit.

Caltech is an Equal Opportunity Employer with an Affirmative Action Program. We encourage applications from qualified women, members of minority groups and persons with non-disqualifying handicaps. 6097(A)

MEAT RESEARCH INSTITUTE SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER

to conduct analytical, biochemical and endocrine studies with laboratory and meat animals.

The objective is to define and identify the means of manipulating those sub-cellular mechanisms of sex steroid action, which lead to sex-dependent patterns of somatic development.

Qualifications: Pass degree, H.N.C. or equivalent with experience or an honours degree in biochemistry or physiology. At least five years post-qualifying experience is normally required for appointment to the higher grade.

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H.S.O.—£4,101 to £5,448.

Starting salary depending on experience. Permanent, pensionable post.

Closing date for applications: September 22, 1978.

Application forms: Personnel Officer, Meat Research Institute, Langford, Bristol BS18 7DY. 7052(A)

UNIVERSITY OF SURREY DEPARTMENT OF BIOCHEMISTRY LECTURER IN FOOD SCIENCE

Applications are invited from suitable qualified graduates for a Lectureship in Food Science in the Department of Biochemistry. Preference will be given to applicants with postgraduate experience, and industrial experience would also be advantageous.

Salary will be within the range £3,660 to £7,308 per annum according to qualifications and experience. Superannuation will be under U.S.S. conditions.

Further particulars about the post may be obtained from the Academic Registrar (LFG), University of Surrey, Guildford, Surrey GU2 5XH or Tel. Guildford 71281, ext. 452. Applications from men and women in the form of a curriculum vitae, together with the names and addresses of two referees, should be sent to the above by September 29, 1978. 6068(A)

WELSH NATIONAL SCHOOL OF MEDICINE AND UNIVERSITY OF SUSSEX POSTDOCTORAL RESEARCH OFFICERS

Applications are invited for two research posts, funded by the Medical Research Council, to work on a three-year collaborative project between Dr F. McCapra, Department of Chemistry, University of Sussex and Dr A. K. Campbell, Department of Medical Biochemistry, Welsh National School of Medicine. The project is entitled "Biomedical applications of chemi- and bioluminescence". It concerns the synthesis and biochemical characterisation of the prosthetic group of Ca^{2+} -activated photoproteins, luminescent labels of proteins as a substitute for radioactive labelling and luminescent reagents for biochemical studies in intact cells. Further particulars of the posts are available from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN (quoting ref. No. C38/4/24).

Applicants should have completed their studies for a Ph.D. and preferably have experience in either organic synthesis or biochemistry. Starting salary can be up to £4,130 on Grade 1A for University Research and Analogous Staff.

Applications should be sent by October 1, 1978 both to the Registrar and Secretary of the Welsh National School of Medicine (address as above) and to Dr F. McCapra, Department of Chemistry, School of Molecular Sciences, University of Sussex, Falmer, Brighton, Sussex BN1 2QJ and should take the form of a curriculum vitae plus the names and addresses of two referees. 7012(A)

UNIVERSITY COLLEGE LONDON DEPARTMENT OF BIOCHEMISTRY

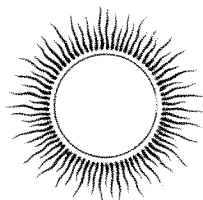
Applications are invited for two LECTURER posts in the Department of Biochemistry. Starting date by mutual agreement. Applicants should be graduates in Biochemistry or Molecular Biology with postdoctoral experience. They should possess the capacity to initiate their own research programmes and be prepared to contribute fully to the teaching programme of the Department. Excellent facilities for research are available. Salary according to age and experience within the range £3,883 to £7,754 p.a. plus £450 London Allowance. Applications including full curriculum vitae, list of publications and names of two academic referees should be sent by the end of October to Professor B. R. Rabin, (N), Department of Biochemistry, University College London, Gower Street, London WC1E 6BT. 7035(A)

UNIVERSITY OF THE WEST INDIES—TRINIDAD

Applications are invited for the post of SENIOR LECTURER/LECTURER in PHYSICS. Must have specialisation preferably in the fields of Optics including Infrared Optics and thin Films, or in High Energy Nuclear Physics, including Plasmas, with expertise on the interaction of radiation with materials. Salary scales: 1977/78—Senior Lecturer TT\$25,827 to TT\$34,017 p.a.; Lecturer TT\$19,071 to TT\$29,799 p.a. (£1 sterling=TT\$4.62) F.S.S.U.: Unfurnished accommodation if available at 10% or furnished at 12% or housing allowance of 20% of pensionable salary. Family passages. Study and Travel grant. Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Secretary, U.W.I., St Augustine, Trinidad as soon as possible. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Further particulars may be obtained from either address. 7009(A)

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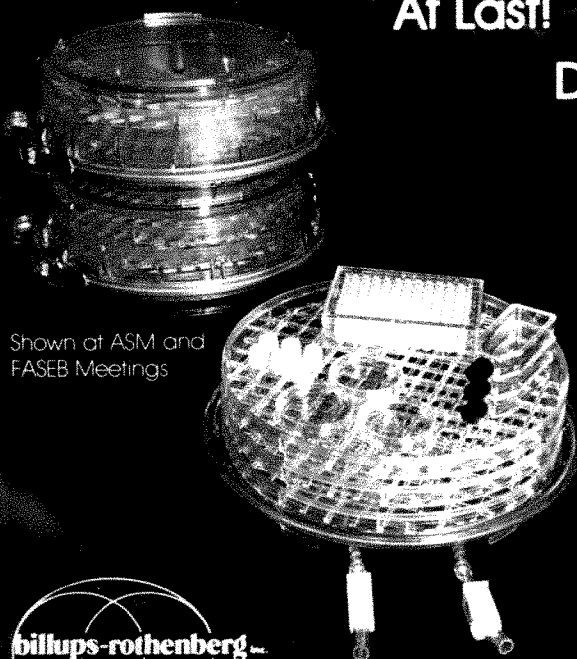
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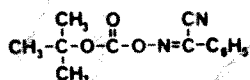
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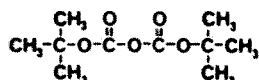
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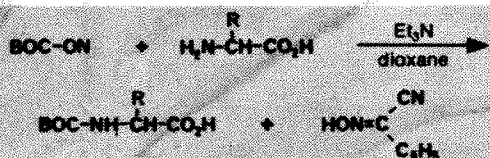


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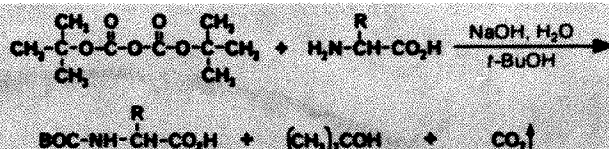
excess of triethylamine at room temperature for several hours. Several *t*-BOC-amino acids prepared in this manner are given in Table 1.³

Table 1

Amino acid	Yield (%)
Gly	87
Leu	72
Met•DCHA	82
Phe•DCHA	98
Thr•DCHA	100
Tyr•DCHA	82

tert-Butoxycarbonylation with **di-*tert*-butyl dicarbonate**^{4,5,6} involves the treatment of the sodium or amine salts of amino acids with di-*tert*-butyl dicar-

bonate at room temperature for 10-30 minutes. Only *tert*-butyl alcohol and carbon dioxide are formed as by-products.



Some of the *t*-BOC-amino acids which have been prepared in this manner are given in Table 2.⁴

Table 2

Amino acid	Yield (%)
Ala•DCHA	84-88
MeAla	74
Val•DCHA	88
(Lys) ₂ Cu	94
(Orn) ₂ Cu	95
Asp(OBzl)	83
Glu(OBzl)•DCHA	86

Aldrich is pleased to offer a choice between two excellent reagents for the introduction of the *t*-BOC protecting group. To each his own!

References:

- 1) J.W. Barton in "Protective Groups in Organic Chemistry," J.F.W. McOmie, Ed., Plenum Publishing Co., Ltd., London, 1973, Chapter 2.
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Cover picture

Solar eclipse viewed from Caracas, Venezuela. The detection of atmospheric gravity waves during a solar eclipse is discussed on page 109. (Photograph: N. Bravo-Franco & E. Yezpez)

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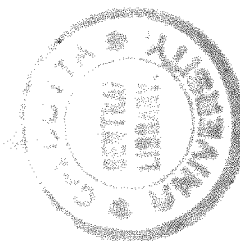
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nature

14 September 1978

Why go to the British Association?

IN many ways the annual meeting of the British Association for the Advancement of Science, just concluded in Bath, was quite a success. There was an excellent turnout of members of the young scientists' wing of the organisation—several hundred students in the 11-to-18-year-old category were regular attenders at the meeting and seemed both to enjoy themselves and learn a lot. Audiences were supplemented by the usual senior contingent of enthusiasts for science, some with a professional interest in science, others keen amateurs. A wide variety of lectures was available, including, as is customary, sections on geography, sociology and economics. Symposia on broad topics such as national food requirements, science and technology for development and decision-making in science drew reasonably good crowds. The only thing missing was a reasonable turnout of the research scientists—academic, industrial and governmental—whose work the association attempts to portray.

Certainly there were nearly three hundred speakers in the four days of the meeting, and most of these were actual researchers themselves. But many of these stayed long enough to deliver their wares and no longer. The number of those actively involved in research (outside the officers of the sections) who gave the meeting four, three or even two days of their time was exceedingly small.

There are some very sound reasons for this: the meeting comes in the midst of university vacations; there are other more specialised conferences that have to be attended; there is a limit to the number of conferences any one scientist can be expected to attend, and (a circular and slightly less sound argument) there are not enough like-minded people around to warrant staying any length of time.

Of course the meeting is not a high-powered affair and there is no obvious immediate research benefit

from attendance, but are the channels by which scientist can speak to scientist across the disciplines so good elsewhere that the British Association can be ignored? For one of the most widely remarked features of science is that significant advances frequently occur when people from one discipline discover intellectual sustenance in another, maybe remote discipline. The chance to make new contacts over a period of a few days seems just the sort of 'removal of those disadvantages that impede scientific progress' which the founders of the association aimed at.

But there is another more selfless reason why research workers should attend the annual meeting more consistently—the good effect they can have on the enthusiastic young contingent. Whilst it is not necessary that every lecture delivered to the association be comprehensible to a 16-year-old, many of these young scientists would value relaxed conversations with people whom they obviously respect. Such conversations would help them understand just what it is like to be a scientist and which particular fields of study might appeal to them. The profession of research scientist is not easily understood from outside and is subject to many misapprehensions; evenings of chat in coffee bars are probably as good a way as any of dispelling illusions and clarifying ambitions.

If there was one other disappointment in the attendance at Bath it was the poor, almost negligible turnout of those who are involved in big decisions which can have a scientific content. One would have thought that many politicians, top civil servants and leading industrialists would use such an occasion to keep well informed and to sound out their constituencies. Not so. This is done in comparable assemblies in some other countries, but not in Britain. The result is a narrow-mindedness and compartmentalisation that seems particularly severe in British public life.

A. J. V. Gale 1895-1978

ARTHUR GALE, joint Editor of *Nature* from 1939 to 1961 and associated with the journal since 1920, died on 4 September 1978.

Educated at Selwyn College Cambridge, he was involved in military service for most of the First World War, and was then persuaded by Sir Richard Gregory, recently appointed Editor, to join *Nature* as Assistant Editor. In 1939, on Gregory's retirement, the editorship passed into the joint hands of Mr Gale and Mr L. J. F. Brimble, who had come to *Nature* in 1931. Brimble continued after Gale's retirement to edit the journal single-handed until his death in 1965.

In order that Gregory and later Brimble should lead a lively public life, attending conferences, meeting scientists, championing causes, it was necessary that someone of scrupulous habits should be back in the

office ensuring that the journal appeared on time each week, and that the appropriate bread and butter of *Nature* was attended to. This Gale did to perfection. He knew exactly what *Nature* was about, and he understood the science well, but his responsibility was not to comment or interpret but to ensure that other people were free to do so. He was essentially the servant of *Nature* and thereby of the whole scientific community.

He claimed that he only ever wrote two leaders, yet paradoxically they were at a most momentous time for science's relation with society: the two weeks following Hiroshima and Nagasaki. His calm and sensible words at that time made one regret that he did not write more often.

He married Gwendoline Veysey in 1929 and to her and to his son and daughter we send our sympathy.

Biologists urge US endowment for conservation

One in five animal species may be extinct by the year 2000, writes **John Douglas**

AMID dire predictions about the future of tropical forests and endangered species, an international group of biologists has called on the United States government to establish a national endowment, similar to the national endowment for the arts, "to support original research, both theoretical and applied, in the area of conservation biology". The conference also called on governments and private groups of all developed nations to offer their support to establish and protect "some remnants of natural habitat in tropical countries". Both resolutions were adopted by acclamation at the end of a four-day conference on conservation biology at the University of California, San Diego, last week.

In recommending establishment of the new research endowment, the conference members cited the present lack of any appropriate US agency to fund and coordinate research in environmental management, wildlife biology and conservation. "In particular, we need an agency to fund research relevant to the stewardship of the Earth's remaining natural diversity". Specifically, the resolution calls for increased support for breeding programmes to help save endangered species, research on the ecology of endangered habitats, and an inter-disciplinary approach to the biology of conservation, environment, and animal populations.

To help preserve remaining tropical habitats, the conference again called on the US government to take the lead by launching a major programme "to aid underdeveloped nations financially in establishing and protecting national parks and biological reserves". Otherwise, the resolution concluded, destruction of tropical forests may seriously affect climatic stability, soil erosion, water quality and economic development, "generally impoverishing human existence".

The plight of tropical forests was detailed in a paper by T. C. Whitmore of the University of Oxford. "The second half of the 20th century will stand in history as the brief period during which man reduced the area of the world's richest and most complex ecosystems to about one-third of their potential area", he said. "An onslaught has developed on tropical moist forests which is predicted to continue to accelerate to reduce them to scattered fragments by 2000 AD."

Between now and the turn of the century, the demand for tropical hardwood log production is expected to triple, said Dr Whitmore. That will

require cutting 556 million hectares of forest, leaving only 30% (480 million hectares) of the total area under virgin conditions. Furthermore, the process of using bulldozers to retrieve the logs may compact as much as 70% of the soil in affected areas, hindering forest regeneration. "The prospect is frightening" he concluded.

Even if large national parks and game preserves are established, however, the loss of the great tropical forests will halt the further evolution of most tropical vertebrate species and drive many animals into extinction, according to papers delivered by Michael Soule and Bruce Wilcox of the University of California, San Diego. By using species data from island habitats the authors estimated the minimum area required for various animals to continue speciation. Even the largest of today's national parks are too small by an order of magnitude to foster continued evolution of large mammals, they conclude.

As a result of inbreeding and environmental accidents, Soule and Wilcox predict that the animals trapped in reserves too small for their continued evolution will begin to die out. Taking 19 East African national parks

as an example, the researchers conclude that 11% of the large animal species will have become extinct in 50 years and 44% will be gone in 500 years, unless new management techniques are introduced. If present trends persist worldwide, only about 1% of the Earth's surface will remain in a preserved condition by the turn of the century, which would eventually lead to the extinction of perhaps 3.5 million of the known 5.0 million terrestrial animal species. One million may disappear just by the year 2000. "The ark is sinking" says Wilcox.

Several speakers addressed the question of how large a population of animals must be in order to avoid eventual extinction, and what breeding practices might be adopted to encourage the vitality of a species. Ian R. Franklin of the Genetics Research Laboratories (CSIRO), North Ryde, New South Wales, estimated that genetic considerations alone would require an "effective population" of at least 500 individuals. (Since not all members of a group of animals contribute equally to the gene pool of the next generation, the number of genetically "effective" individuals ranges between roughly half and three-quarters of the total population.) Below the minimum population, Franklin says, the random loss of desirable traits,

Animal behaviour



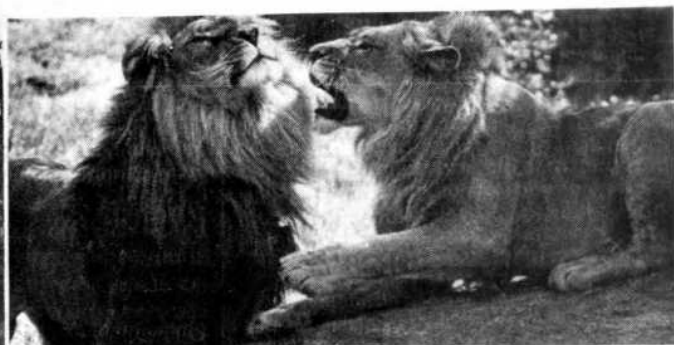
KENNETH MELLANBY

I WAS recently a member of a panel on a radio programme which discussed the topic "Do animals have rights?" The panel included representatives of animal protection societies and a scientist involved in research. The scientist was a well-known journalist and broadcaster. We had a live audience of about 200 and

the programme, with short breaks for advertisements, went on for an hour and a half.

The programme started with the chairperson asking the members of the panel to state, briefly, their views on animal's rights. All agreed that animals should not be made to suffer unnecessarily, but disagreed as to what suffering meant, and on obvious subjects like vivisection, hunting and factory farming. I thought that we should concentrate on man's responsibilities rather than on animal's rights. Domestic animals, cattle and sheep, and pets such as dogs and cats only exist because man breeds them, and controls the numbers which are born. Without man, most breeds would soon become extinct, though in some areas cats have escaped and become pests, endangering the native fauna. As man has created domestic animals, he has the responsibility for providing conditions where they do not suffer—even conditions where we can reasonably imagine that they enjoy life. But if man disappeared from the world, so would most domestic animals.

The situation regarding wildlife is different. Wild animals, for the most



Left: trans-Amaçonia highway and the jungle; right: maned lions each worth half-a-million dollars as Kenyan tourist attractions

called "genetic drift" will make a species unfit for survival.

Much of the problem of establishing and maintaining large reserves is economic: the need is greatest in areas encompassed by the world's poorest nations. William G. Conway of the New York Zoological Society concludes "The preservation of the majority of the animals that man finds attractive probably cannot be sustained on any provable economic basis". However, some nations, he says, have successfully used their preserves to bring in hard currency through tourism. In Kenya's Amboseli National Park, for example, a single maned lion is estimated to be worth \$515,000 as a tourist attraction, compared to only \$1,150 for commercial purposes as a skin.

For those animals that cannot be preserved in the wild, Conway suggests the limited use of zoos as "gene banks"

until future generations can be re-introduced into the wild. With careful mating, the number of individuals needed might be as small as 50 to 100 animals, he says, but even then half their genetic diversity would be lost and only 100 species could thus be maintained if half of all the zoo capacity in the United States were marshalled to the task.

Another way to preserve species by making some of them more profitable to indigenous people was discussed by Malcolm Coe of Oxford. The oryx, for example, is better adapted to life in Africa than cattle, he said, and some domesticated herds of eland, springbuck and greater Kudu have been established. Another advantage of using such wild ungulates for food comes from their high proportion of lean meat — 43% compared to 23% of the carcass of cattle raised under the same

conditions. Even crocodiles might be raised as a valuable cash crop, since their hides bring a high price and they grow much faster in captivity.

The generally sombre message of the conference was phrased in particularly vivid terms by Thomas E. Lovejoy of the World Wildlife Fund. Between 50% and 85% of all species on Earth have yet to be named, he said, and a major fraction of these may pass entirely out of existence still unrecognized. They will be replaced by the quickly evolving, highly productive species that can coexist with man, often as pests. "Cockroaches and Norway rats will loom larger in 21st century bestiaries," says Lovejoy, "as will crabgrass and dandelions in the floras".

Bison trouble

BYELORUSSIA has been facing a somewhat unusual hazard of conservation at the—literally—grass-roots level. The primeval Bielaviezskaja forest is a reserve for, among other species, Europe's last remaining bison. Virtually exterminated during the war, the bison herd is now multiplying in a most satisfactory manner, and it is hoped shortly to remove the bison from the Red Book of endangered species.

In the meantime, however, bison can occasionally cause problems — as certain collective-farmers found this summer. On the "Komintern" collective farm near Barysau, one particular bison claimed as its range a large hayfield, chasing the only farmer who dared approach it up a tall tree.

Denied more "radical means" of evicting it, the Farm Management Board decided to leave the field to the bison — whereupon several other bison moved in. To date no information is forthcoming as to whether the bison have been dislodged.

This is not the first time that forest bison have caused disruption of local life; a few years ago, at Hajdowka on the Polish side of the forest, one of the local bison went to sleep on the railway lines, following this exploit by a visit to the local school.

Vera Rich

part, only continue to exist where man has left conditions—habitats—suitable for them. As a conservationist I think that we should do more to preserve and develop such habitats, even though this conflicts with the needs of agriculture to produce food for the growing human population.

Although the producer of this radio programme had tried to provide a balanced audience, and had invited farmers, those engaged in animal experiments, and sportsmen to participate, these groups were greatly outnumbered by those who considered themselves to be animal lovers. There were colourful and hirsute young men and drab girls wearing T-shirts proclaiming them to be hunt saboteurs. We had anti-vivisectionists of all persuasions, vegetarians, some milk drinkers, some vegans, and some who seemed mainly to wish to oppose the politics of the establishment. Although there were good questions and sensible comments from the floor, the majority of the audience had not come to listen or to discuss. Their purpose was to stop those with whom they disagreed from speaking.

Thus when one panel member tried

to give the correct breakdown of the number of animal experiments in Britain, showing that these were mostly for genuine medical tests and not just to swell the profits of cosmetic manufacturers, a section of the audience, largely consisting of remarkably unattractive women resembling those who knitted when the guillotine functioned in revolutionary France, bayed "Lies, lies, lies, sit down, we won't listen to your lies". When I was asked about the policy of culling red deer in the Scottish Highlands, and said this was necessary to keep the population healthy, as otherwise, as wolves and other predators which controlled numbers were no longer present, the deer would increase until many died from starvation and disease, someone shouted: "Why not cull human beings?" The noise made by the audience was quite alarming, and made the interruptions heard at question time in the British parliament sound almost human.

I am afraid that my main reaction to the evening's entertainment was to wonder why those who profess to love animals hate their own species so much!

Science at the UN: coordination or chaos?

Peter Collins reports on the likely demise of the UN's only scientific advisory body, 'ACAST's. Who will replace it?

AT least we should be represented at our own funeral" remarked the chairman of ACAST's recent meeting in Geneva. For this, the United Nations Advisory Committee on the Application of Science and Technology, is almost certainly one of the bodies likely to disappear in the restructuring to which its parent body, ECOSOC (the Economic and Social Council) is now being submitted. In this process ACAST has no say, and may find few friends to speak up on its behalf: government delegates as a whole, and especially those of the developing countries who hold a voting majority, do not like independent committees. Be that as it may, the abolition of ACAST at the present juncture would be most unfortunate. Its recent meeting was one of the liveliest and most useful since its foundation, and its discussions had an air of realism and urgency often lacking in past. This was perhaps due to the presence of several new members of this individually selected body, invited to replace some of those who had given it the reputation of representing the scientific establishment, with few original ideas and, at times, little apparent awareness of the trends and possibilities of modern science and technology.

In search of harmony

One of the major subjects discussed was the matter of policy for science and technology within the UN system itself. ECOSOC went on record some time ago to the effect that "the planning of activities in the various organisations of the United Nations should be harmonised and gradually integrated into a United Nations science and technology policy".

However, the idea of a centrally-controlled, general policy for science and technology within the system is widely considered to be impracticable, even if indeed it is desirable. What is seen as a reasonable objective is the harmonisation of policies within and between the various parts of the system. Some decisions on this subject are expected to form part of the Programme of Action for UNCSTD next year, and some sort of closer coordination in this field is also seen as essential for the evolution of the "new international economic order", in which the whole UN system and its member governments are now involved.

When it came to the means of bringing about such harmonisation, ACAST considered three main possibilities. The

first of these was creation of a new agency charged with the overall coordination of science and technology for the entire system. ACAST commented that "experience in the United Nations (as well as with almost all organisations) is that new agencies become concerned not only with their own survival but also with increasing their influence and activities."

The second possibility, the suggestion of setting up an organisation for science and technology equivalent to the UN Environment Programme, was looked on somewhat more favourably. But to be effective it would sooner or later require, as has UNEP, a fund, and it is unlikely that member governments would agree to the creation of yet another large fund with all its administrative costs and the need for constant checks on the validity of the operations in which it might become involved. In any event, such a programme would have to rely on inputs from the rest of the system, and would need to have sufficient political or other support "to ensure consistency among the various agencies objectives and strategies through its decisions regarding funding and through coordination".

Much the most attractive, at least under present circumstances, would appear to be the third possibility, defined as a Centre for Concerted Action and Coordination on Science and Technology. Its purpose would be to systemise and synthesise information on all science and technology programmes and strategies throughout the system; to keep the Secretary-General advised of what was going on and what were the objectives and sub-goals of the other organisations within the system, relating these to the overall objectives; and to maintain contacts with the scientific and technical community, including intergovernmental and non-governmental organisations.

Questions involving research could be referred to other organisations within or outside the system, or, suggest ACAST, "to a committee of no more than twelve top-level experts or to such *ad hoc* advisory committees or working groups as might be necessary". Such a centre could create the setting for some sort of planned policy-making for science and technology within the system, besides providing a mechanism for coordination and harmonisation. Because it need have only a small staff, it could be flexible and could respond quickly to changing situations and in

providing advice to the Secretary-General. At the same time, it would compete with no existing organisation for either resources or authority.

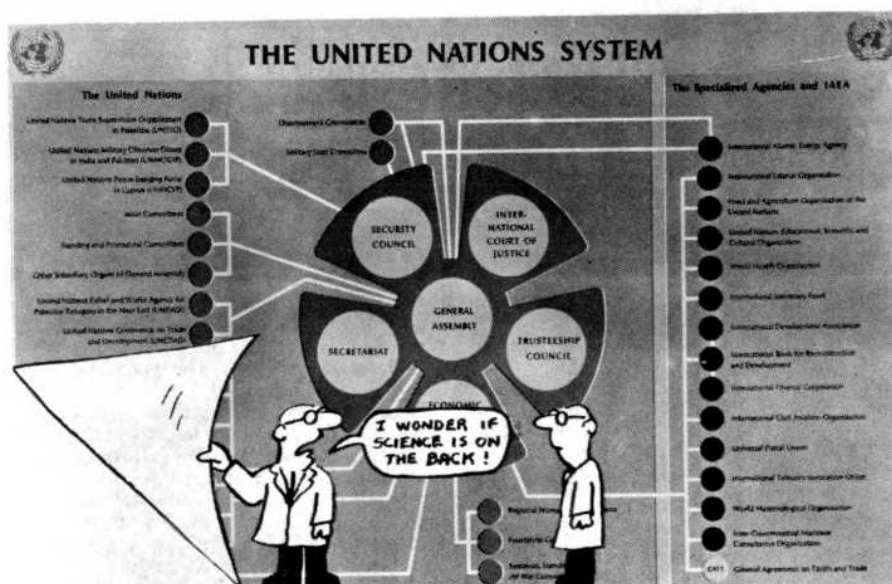
If set up at the present juncture, such a centre could be designed to allow for the present restructuring of the various organs involved in ECOSOC and in the central control of the UN itself. Nonetheless, it would still have to face the problem raised by the specialised agencies which still seem set against any form of coordination, let alone integration, as was also made clear at the ACAST meeting.

A message for UNCSTD

From the immediate point of view of the scientific community outside the UN, the most interesting discussions concerned the meeting to be held in Vienna in the week immediately preceding UNCSTD, 13-17 August, 1979. Known hitherto as "Forum A" (to distinguish it from "Forum B", the non-governmental event being held parallel with UNCSTD and organised by the Austrians themselves), this is now the responsibility of ACAST, and will probably be called the "ACAST Colloquium on Science, Technology and Society". It will be confined to some 200 invited scientists, technologists and other persons, including governmental representatives and those of interested organisations outside the system. Provision is being made for science writers and other journalists and possibly, if space allows, for members of the public. This is intended as a serious scientific meeting, the output of which will include a message from the world scientific community to UNCSTD, as well as proposals relevant to the conference programme of action.

Besides various papers to be prepared by the UN Office of Science and Technology, which is providing the secretariat for this meeting, there will be important inputs from four symposia being held within the context of UNCSTD between now and next summer. These include that at Tallin, on Trends and Prospects with regard to the Development of Science and Technology (4-8 December 1978); Science and Technology for Development (Singapore, March 1979) organised by a group headed by ICSU; Technology for Development (Abidjan, Ivory Coast, April 1979); and Science and Technology in Development Planning (Mexico, May 1979).

Priority among subjects discussed at the present ACAST meeting went to those on which the committee's views had been requested by José da Costa, Secretary General of next year's UN Conference on Science and Technology



for Development (UNCSTD). Insofar as the conference itself is concerned, the most important of these was the discussion of "obstacles to the application of science and technology for development". Having as a basis a list of 31 general areas in which such obstacles might arise, ACAST pointed out that this list could be expanded to a hundred or more. It considered them by categories, each of which might be susceptible to the same type of solution at national, regional, or international level. They then fell into two broad groups: political, financial, economic and institutional obstacles to development; and those involving education and human resources, the availability and dissemination of information, and the psychological problems inherent in any society subject to the pressures inseparable from rapid development.

ACAST's suggestions stress the need for an integrated approach to specific problems that are seen as capable of reasonably early solution. Thus, to give a few examples: to improve the communication gap in a given country between its scientific and technological community, decision-makers and users, a well-mandated national advisory council might be set up, that would monitor trends and developments and interpret them to the leadership, as well as interpret political goals and objectives to the scientific and technological community. At the regional level, programmes of common interest could be identified as part of regional economic planning policy, with a regional machinery to coordinate and evaluate their performance. At a practical level, regional servicing facilities might be established, to maintain and service the special equipment of all developing countries in the region; it could maintain a spare parts bank to avoid the harrassment of delays in obtaining equipment spares. With regard to in-

formation at both regional and international levels, there should be a network of information systems not only to act as a data bank, but also to assist in collecting trends of development in technologies and to interpret their implications for developing countries.

ACAST concluded that "at the national, regional as well as international levels, the psychological obstacles can only be removed by redesigning and strengthening the education, training and information systems".

Recognising social science

Finally, ACAST briefly discussed its own future. Obviously, if something like the UN Centre for Concerted Action and Coordination on Science and Technology described above is set up, there would be a function for a small body similar to ACAST, and this is already foreseen in the suggested "committee of top-level experts". One point of which ACAST is now well aware is the need to include, in whatever body replaces it after UNCSTD and as a result of the restructuring exercise, representatives of the social sciences. The intention was never to include these when ACAST was set up in 1964, but their presence is at last seen as essential if a balanced view is to be obtained of what science and technology are doing, can and should be able to do, in the world as a whole. This much is now recognised. But whatever the decisions of the General Assembly, which has the final say in ACAST's future, it is certain that some such body must exist if the UN Secretary-General, essentially preoccupied with political affairs and the infighting of the UN system, is to be kept aware of what is happening in the science and technology on which the world increasingly and inevitably now depends. □

Racial resegregation measured

DESPITE decade-long efforts to integrate schools, increase individual welfare payments and revitalize central cities, the underlying conditions attacked by these programs remain almost unabated, according three reports delivered at last week's meeting of the American Sociological Association in San Francisco.

A new study of school integration and the so-called "white flight" of middle class caucasian families to suburbs from the city centres reestablishes the seriousness of this problem, first treated in a controversial report by James Coleman in 1975. Coleman's treatment was severely criticized at the time for neglecting concurrent demographic changes and not distinguishing between forced and voluntary desegregation.

Now, David J. Armor of the Rand Corporation, Santa Monica, California, has analysed integration statistics by estimating what out-migration of whites would have taken place anyway, taking into account the different birth rates between whites and blacks, and comparing the results of court-ordered busing to voluntary methods of desegregation. He concludes that forced integration accelerates white flight by a factor of from two to four, but that voluntary integration may not accelerate it at all.

He compares specific cases by using a "desegregation index", defined as the average percentage of white students in schools attended by minority students. (If minority students were divided among all American schools completely randomly, each school would have a desegregation index of 70 to 80. The smaller the index, the less integration.)

Armor found that, with the beginning of court-ordered integration, the index for a particular school district usually jumps. In one year, the desegregation index of Pasadena, California, rose from 37 to 53. But over the next seven years, it steadily dropped back to 35, in 1977. By comparison, nearby San Diego started its voluntary busing plan in 1968 with an index of 43. The index rose gradually to 46 and has now slipped back to 44.

During roughly the same period, a variety of programs were introduced to help raise the average income of blacks and other minorities. A report by Robert B. Hill of the National Urban League Research Department shows that again many early gains have been eroded by the "benign neglect" policies of the Nixon and Ford Administrations and the two severe recessions of the early 1970s.

John Douglas

correspondence

Sunspots and flu: a correlation

SIR,—Seasonality exerts a profound but ill-understood influence on epidemic influenza. North of the tropic of Cancer and south of Capricorn epidemics usually break out in winter when the relevant portion of the globe of the Earth is most distant from the Sun. The purpose of this note is to call attention to a longer-term association of type A influenza with solar activity that has become apparent since human influenza virus was discovered in the epidemic of 1932–33, namely that the periods of world-dominance of successive major subtypes of influenza A virus have synchronised closely with the periodicity of sunspots (see Figure). The two sets of cycles are probably synchronised at least as far back as 1917.

Influenza pandemics have occurred in the twelve months after type A influenza virus has undergone an antigenic shift leading to a new major subtype. The new major variant with altered haemagglutinin (H) and neuraminidase (N) finds the population inadequately protected by influenza caused by its predecessor. The change has enabled it to bypass their immunity. The predecessor promptly disappears and the new major variant with its minor varieties dominates the world causing all the type A influenza for the next decade or so until itself displaced by the next antigenic shift. The major subtypes since 1933 have been identified serially according to H & N composition as H_0N_1 viruses isolated before 1947, H_1N_1 1947–1957, H_2N_2 1957–1968, H_3N_2 1968–1978. Each major subtype completely disappeared at the next antigenic shift except that H_1N_1 viruses reappeared in many parts of the world in 1977 and are still (28 July 1978) causing influenza concurrently with H_3N_2 viruses. The pandemic of 1918 is usually attributed to antigenic shift producing the major subtype HSw_1N_1 . A small outbreak caused by virus of this major subtype occurred in 1966.

Sunspots undergo cycles of activity varying from seven to seventeen years,

average eleven years. Activity increases to maximum more rapidly than it declines to minimum, and maxima sometimes stand upon a plateau of several years of approximately equal activity. The true solar sunspot cycle consists of two consecutive cycles of minimum to minimum activity (see Gibson, Edward G. *Rev. Geophys. Space Phys.* 10, 395–462; 1972). Antigenic shifts of influenza A virus to H_1N_1 , H_2N_2 and H_3N_2 coincided with sunspot maxima of 1947, 1957 and 1968 respectively. The sunspot maximum of 1937 coincided with severe and widespread influenza, but no antigenic shift was recorded. The sunspot maximum of 1928 coincided with a pandemic which may have signalled the antigenic shift from HSw_1N_1 to H_0N_1 virus. The previous sunspot maximum of 1917 anticipated the pandemic of 1918–19. Fascinating as it is to attempt to investigate the association throughout the centuries of sunspot records, influenza records are probably too dubious to make the attempt worthwhile. The next sunspot maximum is however to be expected shortly, perhaps in 1979. The behaviour of type A influenza virus during the next few seasons will be watched with keen interest tinged with apprehension.

Yours faithfully,

R. E. HOPE-SIMPSON

Epidemiological Research Unit,
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The Beacon containing an interview in the form of questions and answers between someone described as a *Beacon* reporter and someone *The Beacon* describes as Professor Eysenck. Could the granting of this interview, by one who gives so many, have slipped his mind?

However, the most serious problem with the answers to my letter (*Nature* 274, 738; 1978) is that both Professors Eysenck and Jensen fall yet again into the typological trap of the confusion of social and biological categories. To speak of "racial mean differences" to is do the reverse of to "treat each person individually", which Professor Eysenck claims he wants; it is to categorise them as belonging to a group, which although socially defined (eg, Black, Jewish) Professors Eysenck and Jensen regard as having biological meaning. The resulting scientific errors which flow from their attempts to biometrise group characteristics are apparent to most biologists. As the National Front propaganda has shown, racist ideology feeds on this biologising thinking, and it needs firm public rebuttal.

Yours faithfully,

STEVEN ROSE

Open University,
Milton Keynes, UK

Racism

SIR,—Professor Eysenck's clear dissociation from the National Front and other 'explicitly' racist organisations is to be welcomed, and I assume that he will be taking steps to ensure that the organisation and its associated journals cease using his name. In the meantime, although I am sure that when he denies that he has given an interview to *The Beacon* he does so in good faith, a further clarification seems required, as I have in my possession a xerox copy of an issue of

Origins of "ecology"

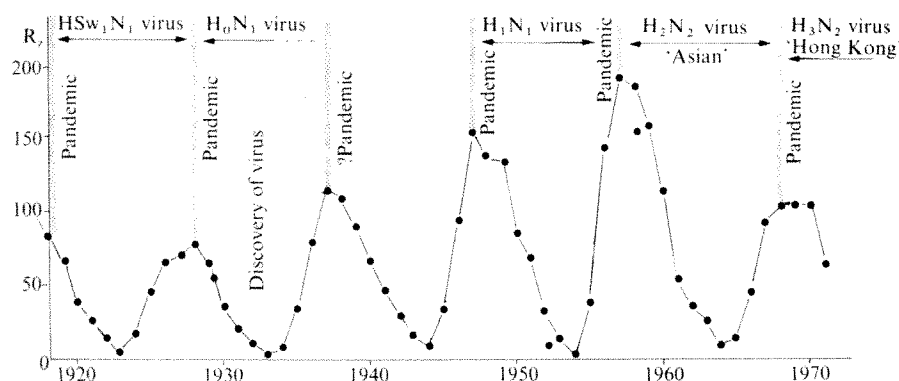
SIR,—In reviewing the book by D. Worster, *Nature's Economy*, Kenneth Mellanby (*Nature*, 6 April 1978) states that Haeckel "is believed to have coined the word [ecology] in 1873". I do not know if the word was used earlier, but it is certainly true that Ernst Haeckel in his volume, *Generelle Morphologie der Organismen*, Berlin, 1866, Vol. 2 ("Allgemeine Entwicklungsgeschichte der Organismen"), p 286, states: "Unter Oecologie verstehen wir die gesamte Wissenschaft von den Beziehungen des Organismus zur umgebenden Aussenwelt". He therefore gives the correct definition of ecology, as we understand it today. Haeckel goes on (p 287): "Dagegen hat sie die Beziehungen desselben zur Aussenwelt, die Stellung, welche jeder Organismus im Naturhaushalt, in der Oeconomie des Natur-Ganzen einnimmt, in hohem Grade vernachlässigt".

Haeckel's definition of ecology, integrated with the concept of ecology as the study of the "economy of nature", is repeated in Haeckel's popular collections of lectures, *Natürliche Schöpfungsgeschichte* (1868), and *Anthropogenie* (1874). Both books were translated into many languages, and had dozens of editions, containing increasing numbers of 'lectures'. Readers may like to refer to a short history of the concepts of 'ecology' and of the 'economy of nature' in my paper: "Ecologia e economia", *Giornale degli Economisti*, 32, 435–455 (1973).

Yours faithfully,

GIORGIO NEBBIA

Università di Bari, Italy



Sunspot cycles and major variants of influenza A virus. Changes of major variant occurred abruptly with disappearance of predecessor variant. R_z : Zurich yearly means of daily Relative Sunspot Numbers

news and views

Mussel watching

from B. L. Bayne

BIVALVE molluscs, particularly mussels and oysters, are valuable indicators of pollution in coastal marine environments and estuaries. Among their qualifications for this role are their sessile mode of life as adults and a capacity for accumulating contaminants in their tissues to levels considerably higher than in seawater. The first attribute makes escape from various environmental stresses impossible. The second makes bivalves attractive to analytical chemists; whereas most pollutants may be present in seawater in concentrations between 10^{-11} and 10^{-12} g g⁻¹, concentrations in bivalve tissue may vary between 10^{-8} and 10^{-4} g g⁻¹. Mussels of the genus *Mytilus* fit this role of indicator organism particularly well because they provide convenient amounts of tissue for analysis and because they are common members of benthic (often intertidal) communities on hard substrata, with a wide and circumpolar distribution in boreal and temperate waters of both northern and southern hemispheres.

In 1975 Ed Goldberg, of the Scripps Institution of Oceanography, proposed a 'mussel watch' in which collections from many sites would be analysed regularly for their body-burdens of heavy metals, halogenated hydrocarbons, petroleum hydrocarbons and transuranic nuclides. The results would provide "a continuing revelation of how man's activities are altering oceanic composition" (*Mar. Poll. Bull.* 6, 111; 1975). In 1976 this idea bore fruit with a surveillance programme in the USA, under contract with the US Environmental Protection Agency. Samples of mussels or oysters are taken from 107 stations on the West, Gulf and East coasts of the US and analysed in five laboratories, each main class of contaminant being treated independently by two analytical groups. The results of the first year's work have now been published by Goldberg *et al.** The report

includes a discussion of sampling strategies, of some of the problems associated with seasonal and size variability, and of the economics involved in sampling and analysis, as well as providing detailed tables and discussions of the results.

In projects of this kind care is needed in designing the sampling procedure in order to ensure true compatibility between sites. Ideally, animals should be taken at a standard size, since the accumulation of metals at least, is known to be size dependent (Boyden *J. mar. biol. Ass. U.K.* 57, 675; 1977). Sampling should be limited also to a standard tidal level on the shore and to a particular season of the year (Phillips *Environ. Pollut.* 13, 281; 1977). When the project covers a very wide geographical area, these may be difficult constraints to meet. Goldberg *et al.* sampled monthly at two stations and found no significant seasonal trend in concentrations of heavy metals or halogenated hydrocarbons; although a seasonal change in ^{239,240}Pu and ²⁴¹Am was interpreted as due to a change in the environment rather than a seasonality in the physiology of the animals. There have, however, been other reports of seasonal changes in the contamination of mussel tissue, a result to be expected knowing the large seasonal changes in metabolism that occur in bivalves associated with gametogenesis and the availability of food. In a widely based mussel watch it would be wisest to include independent checks for possible seasonal effects.

Another problem is posed by the treatment of the samples before analysis. Mussels feed on suspended particulate material (Goldberg *et al.* suggest that this is the main route for contamination by radionuclides) and, particularly in estuaries, their stomachs are likely to contain much inorganic material that would normally be passed out in the faeces. Consequently, a period of 12-48 h spent in clean seawater between sampling and analysis, in order to clear the gut by defaecation, is generally to be recommended.

But this is not practicable in a project on the scale of the US mussel watch, where samples were frozen on dry ice immediately following collection.

Another potential problem, that of calibration between laboratories, was specifically addressed in the US mussel watch, and the results are generally encouraging. Agreement between analytical laboratories was satisfactory for most of the metals and for the transuranic nuclides. Consistent differences occurred between laboratories in the analysis of the two principal degradation products of DDT, p,p'-DDE and p,p'-DDD. These differences are not viewed as alarming, particularly since all samples were sent for analysis to each laboratory chosen to analyse the particular contaminant. However, the need for careful intercalibration studies in future programmes is emphasised.

Having measured the concentration of each contaminant in all samples, a decision on whether the environment is 'polluted' must be made. It is on this point that some argument is to be expected. Goldberg *et al.* recommend that order of magnitude differences (or variations over time) should be considered significant when considering metals or halogenated hydrocarbons. On this basis particular pollution 'hot-spots' are recognised, for example the New York-New Haven area for metals and numerous sites of PCB pollution on the East coast. For PCB 1245, relatively unpolluted areas show tissue levels less than 50×10^{-6} g g⁻¹ dry weight, whereas polluted areas have levels as high as 600×10^{-6} – 800×10^{-6} g g⁻¹. This criterion of an order of magnitude difference in order to discriminate between polluted and unpolluted sites is conservative, but rigorous enough in the context of the US mussel watch data; how generally applicable it may be remains to be seen. In setting such a criterion, however, the need for samples from a large number of sites is emphasised. As Gold-

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*Goldberg, E. W., Bowen, U. T., Farrington, J. W., Harvey, G., Marth, J. H., Parker, P. L., Risenbrough, K. W., Robertson, W., Schneider, E. & Gamble E. *The Mussel Watch, Environ. Conser.* 5, 1-25 (1978).

berg *et al.* point out, some locally high values for metals, in particular, may be due to natural estuarine run-off (see Bryan & Hummerstone *J. mar. biol. Ass. U.K.* **58**, 401; 1978) rather than industrial pollution. This is one aspect of the perennial problem in pollution monitoring, the setting of natural 'base-lines' for particular contaminants. Only by treating many samples from a large number of sites can a real appreciation be gained of true minimal concentrations of pollutants, and this must be particularly true in the coastal waters of industrialised states.

Using these and other criteria, the US mussel watch has identified certain areas of pollution; some of these conclusions came as no surprise, others remain more of a mystery. The study demonstrates the usefulness of the concept and argues strongly for an even wider geographical coverage. Indeed, Goldberg's original aim was for a global marine monitoring programme. The interest already shown by some countries (the proposed Mediterranean mussel watch, for example) suggests that an increase in geographical scope can be achieved.

This prospect poses a challenge for the biologist. Measuring the levels of

contaminants in the tissues of molluscs does provide information on levels of contamination of the water, but the question naturally follows: "What is the biological impact of these pollution loads?" To some extent, chemical studies of pollution require biological confirmation, for it is in the intensity of the effect on the biota that questions of pollution acquire environmental realism. Means of assessing the biological effects of pollutants are gradually being made available (*On the feasibility of effects monitoring, Co-operative Research Report No. 75*, ICES; 1978) and they vary from measures of biochemical response through indices for assessing the physiological condition of individuals to attempts to relate components of animals' responses to the possibilities of lasting ecological damage. Much of this research is focused on marine bivalve molluscs for the same reasons that render these animals attractive to analytical chemists. In promoting a wider application of the mussel watch concept, therefore, attention should be given to the incorporation of measures of biological impact. Goldberg *et al.* comment briefly on this point in their article, but it is a challenge to which the biologists need to respond. □

Large-scale population studies have clearly shown that a disproportionate number of individuals with particular HLA alleles develop certain diseases. Putative disease-susceptibility genes are spread all along the HLA complex, but the closest associations are with alleles at the D locus. For example, Dw2 is increased in multiple sclerosis, and Dw3 in a number of different autoimmune diseases, including thyrotoxicosis (Graves' disease), spontaneous adrenal insufficiency (Addison's disease), and pernicious anaemia. These same diseases show a significant but somewhat reduced association with one of the antigens in the B series, B8. This finding is most easily explained by assuming that B8 and Dw3 do not sort randomly; that is, are in linkage disequilibrium. As I. Roitt (Middlesex Hospital, London) pointed out, rheumatoid arthritis is unusual in that it associates with Dw-4 and shows no significant affinity for antigens of the A, B, or C series. Although statistically significant, these HLA associations are still distant enough to indicate that other genes and non-genetic factors greatly influence the development of autoimmunity. For example, genetic disorders in the particular target organ or sex-associated factors (these diseases are almost all more common in females than in males) are probably involved.

Sometimes HLA typing helps to distinguish different varieties of what was thought to be the same disease. Two different forms of diabetes mellitus can now be distinguished by HLA typing. The so-called juvenile-onset or insulin-dependent form of diabetes is associated in Dw-3, while individuals without this genetic marker are more likely to develop a late onset form of disease that can be controlled by diet or oral hypoglycemic agents, not requiring insulin. As J. Irvine (University of Edinburgh) emphasised, distinction between the two forms of diabetes should now be made, not on the basis of age of onset, but on HLA type and immunological response to pancreatic islet cells. HLA typing is also useful in prognosis; for example, those patients with Graves' disease who are HLA-B8 are less likely to undergo spontaneous remission than patients lacking this trait. I. Mackay (Hall Institute, Melbourne) distinguished two forms of chronic active hepatitis on the basis of immunological response. The autoimmune type of the disease shows an increase in HLA-A1, B8 and Dw-3. No such HLA association is found in chronic active hepatitis patients who are positive for Australia antigen.

Autoimmunity revisited

from Noel R. Rose

It is now some 25 years since autoimmunity emerged as a plausible cause of a number of mysterious human diseases. But, despite the demonstration of autoantibodies in patients' sera or localised in affected tissues, there has been little understanding of how the autoimmune process gets started. The initiating event in human disease is certainly not deliberate injection of autologous antigen mixed with a suitable potentiating agent like complete Freund adjuvant, as it is in experimentally immunised animals. It now seems likely that one of the important prerequisites of autoimmune disorders in the human is genetic predisposition.

Early evidence that genetic abnormalities predispose to human autoimmune diseases stemmed mainly from two kinds of studies. Astute clinicians noticed that certain autoimmune diseases cluster with unexpected frequency in some families. When objective markers of autoimmune disturbance became available in the form of tests for autoantibodies, a statistically significant increase in auto-

immunity was documented even in clinically unaffected relatives of autoimmune patients. A recent line of evidence to support a genetic predisposition towards autoimmunity has come from investigations relating autoimmune diseases (as well as quite a number of non-immunological diseases) to genetically determined cell surface markers, especially HLA alleles. While rarely firm enough to be of practical diagnostic value, these statistical associations point to the existence of disease-susceptibility genes.

The World Health Organization Collaborating Center for Autoimmune Diseases, located at Wayne State University, sponsored a Workshop on Genetic Control of Autoimmune Disease in July 1978.* The purpose of the meeting was to see if the newly generated information derived from genetic investigations might open novel approaches to the diagnosis and treatment of human autoimmune disorders.

To begin, P. Terasaki (University of California, Los Angeles) and A. Svejgaard (State University Hospital of Copenhagen) analysed the voluminous evidence associating autoimmune disorders with particular HLA alleles.

*The meeting was organised by N. Rose, P. Bigazzi and N. Warner. The full proceedings are being published by Elsevier-North Holland.

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The most likely role of HLA in determining susceptibility to disease is through immune response genes that determine recognition of a particular antigenic determinant. Evidence for this concept is based mostly on the close resemblance of HLA to mouse H-2, which is known to control immune responses.

Among the models available for the study of spontaneously appearing autoimmunity in animals, the New Zealand (NZ) strains of mice have been by far the most profitable. The NZB strain spontaneously develops haemolytic anaemia, and the hybrid obtained by crossing NZB with the apparently normal NZW strain results in an autoimmune disease closely resembling human systemic lupus erythematosus (SLE). Extensive efforts have been made to sort out the several genetic factors controlling these autoimmune disorders, but a clear definition has not yet been obtained. To complicate matters, NZ mice contain abundant type C virus particles. Viral genes code for glycoprotein which closely resembles a mouse glycoprotein, and the possibility was raised that antibody originally induced to viral proteins may cross react with the comparable mouse antigen. However, S. Datta (Tufts University) showed that viral genes segregate independently of 'autoimmunity' genes, removing the possibility of a cause-and-effect relationship between viral infection and autoimmunity.

A more likely possibility to explain the spontaneous development of autoimmune disease in NZ mice is a fundamental disorder in immunological regulation. H. Cantor (Harvard Medical School) demonstrated an early deficiency in the subset of T lymphocytes responsible for immunological equilibrium. These cells, recognised by their antigenic surface markers, Ly 123⁺, produce feedback suppression of antibody production. N. Talal (University of California, San Francisco) further showed that the paucity of suppressor T cells in NZ mice can be at least partially remedied by thymic extracts. Another view of the fundamental NZ abnormality emerged from experiments of H. Morse and T. Chused (National Institutes of Health). B cells of NZB mice show spontaneous polyclonal activation even in the absence of T cells. These findings suggest that NZ mice suffer from an intrinsic lesion in their B cells. There may be a more basic stem cell defect affecting both regulatory T cells and B cells.

In contrast to the multiple autoimmunities present in NZ mice, a single autoimmune disorder can be demonstrated in the OS strain of chickens. These animals spontaneously develop a classical form of chronic thyroiditis. L. Bacon (Wayne State University)

DNA structure and transcription

from Andrew Travers

THE mechanisms controlling transcription during the development of different bacteriophages are extremely diverse. Yet another solution has emerged from the studies of Rothman-Denes and her colleagues on coliphage N4. N4, in common with *Bacillus subtilis* phage PBS2, but unlike all other commonly studied phages, does not apparently require the host RNA polymerase for any viral transcription. Instead the virus contains a virus-coded transcriptase of novel structure. This enzyme consists of a single protein chain about 4,000 amino acids long and is thus one of the largest polypeptides so far identified.

How does this enzyme function? Its equally novel template requirements are now reported in the latest paper from Chicago (Falco *et al.* *Proc. natn. Acad. Sci. U.S.A.* 75, 3220; 1978). *In vitro* N4 RNA polymerase strongly prefers N4 DNA, which it copies asymmetrically, producing transcripts corresponding to those RNA molecules synthesised *in vivo* immediately after phage infection. However, in contrast to other bacteriophages, the enzyme has the virtually absolute requirement that this template be denatured. Another pointer to the unusual nature of the specificity of the enzyme is the observation that *in vivo* N4 RNA synthesis is specifically inhibited by coumermycin, a drug whose target is DNA gyrase, the enzyme responsible for inserting negative superhelical turns into closed circular duplex DNA. These results suggest that DNA gyrase must modify the structure of N4 DNA before it can be transcribed by the virion RNA polymerase. Consequently, the authors propose that superhelical DNA may be the required natural template for the enzyme.

Assuming this hypothesis to be correct, we have to ask how the introduction of negative twists into presumably closed circular N4 DNA

so dramatically promotes transcription. The best current analogy comes from studies on *Escherichia coli* RNA polymerase. Before initiating transcription this enzyme recognises a promoter site extending over a region of at least 40–50 base pairs, and subsequently effects a change in DNA conformation at the binding site, a process termed promoter opening. It is well established that increasing the superhelical density of phage DNA templates substantially enhances the rate of transcription initiation by the bacterial polymerase. This enhancement could result from a direct facilitation of enzyme-mediated promoter opening. Alternatively the introduction of superhelical turns could alter the parameters which the enzyme recognises in the DNA template sufficiently to change the initial affinity of the polymerase for a promoter site.

At present the available experimental evidence does not distinguish between these possibilities. However, Levitt (*Proc. natn. Acad. Sci. U.S.A.* 75, 640; 1978) has recently calculated that supercoiling can, in theory, alter the number of base pairs per turn of the double helix by as much as 5%. If this result is applicable to the real world, it is difficult to escape the implication that the spatial arrangement of the DNA sequence in a supercoiled molecule differs substantially from that in a relaxed one. Consequently unless one invokes the additional *ad hoc* assumption that RNA polymerase possesses a similar molecular flexibility it seems highly plausible that the detail of the initial contacts of the enzyme with a given promoter site will strongly depend on the extent of superhelicity of the template. However, the capacity of the bacterial polymerase for differential promoter recognition can be extensively modulated by factors other than subtle variations in DNA structure. Under these circumstances the N4 virion RNA polymerase promises to provide an attractive simpler system to test for the effects of supercoiling on promoter recognition and opening.

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found that the trait is polygenic but is significantly linked to the B locus (the chicken's MHC) in certain families. In other families the B locus linkage may be obscured due to over-riding effects of other genes. Other genetic defects have been found in the OS thymus and in the thyroid gland itself.

On the basis of the many studies of spontaneously developing autoimmune disease in animals, three types of genetic eccentricity can be recognised. One is an increase in immunological responsiveness, possibly due to the presence of immune response genes within the MHC. Such a

gene is probably present in the OS chickens. A second lesion appears in the thymus and in the regulatory cells developed in the thymus. A third genetic defect probably occurs at the level of the target organ and determines where the particular pathological damage may be found.

Among the models of induced autoimmunity in animals, the most clearly defined is experimental allergic encephalomyelitis (EAE). D. Gasser (University of Pennsylvania) found that in the rat the development of disease is associated with the MHC, but other non-MHC related genetic controls can also be recognised. In mice the situation is even less clear-cut, because natural suppressor cells have a major, and possibly over-riding, influence. In some strains it is possible to detect genetic control by reducing the effect of T suppressor cells with cyclophosphamide treatment (R. Arnon, Weizmann Institute).

Another good model for studying genetic control of induced autoimmune responses is experimental thyroiditis. In mice, where many genetically defined inbred strains are available, Y. Kong (Wayne State University) was able to delineate at least two genes that regulated the response to mouse thyroglobulin. One, located near the K end of the H-2 complex, appeared to be a typical immune response gene. A second-order gene near the D end of the complex modified the immune response so that the pathological damage to the thyroid was heightened or diminished.

H. Wekerle (Max Planck Institute, Freiburg) has developed a method of autosensitising T lymphocytes *in vitro*. His experiments strongly suggest that self-responsive precursor T cells are normally present in the body but held in check by homeostatic immunological mechanisms. Further evidence that self-responsive lymphocytes are normally present to testicular antigens emerged from experiments described by P. Bigazzi (University of Connecticut), who found that vasectomy of rats and mice engenders production of sperm-specific antibody, a response also reported in vasectomised men.

Until recently, most immunologists believed that the lack of response to self-antigens came about because lymphocyte clones that carried receptors to self-antigens were eliminated at birth. The investigations described during the Cranbrook meeting contradicted this view. The data showed that both T cells and B cells capable of reacting with many self-antigens are present and waiting to be triggered. The prevention of autoimmune responses, therefore, requires continuous and active suppression.

Two regulatory mechanisms were

discussed in some depth. The first is the generation of antibodies to idiotypes, as described by H. Wigzell (University of Uppsala). Idiotype determinants characterise antibody molecules and immunocompetent cells of a particular antigenic specificity. Idiotype antibodies are strong candidates for physiological regulators of immune response, as they can control production of antibodies with particular specificities by stimulating or inhibiting idiotype-bearing immunocompetent cells.

Immunological regulation also depends on the balance of suppressive and stimulatory subsets of T cells. The balance depends on the properties of the antigen and the method of administration. R. Swanborg (Wayne State University) found that certain determinants of the EAE antigen favour suppressor cell production, specially if given with incomplete rather than complete Freund's adjuvant. E. Engleman (Stanford University) reported that he could generate *in vitro* a factor from human T cells that suppressed allogeneic lymphocyte interactions. The suppression is genetically

restricted by the D locus of the reacting cells, re-emphasising the crucial role of the MHC in immunological regulation.

To many participants, the meeting at Cranbrook foreshadowed a new stage in the thinking about human autoimmune disease, with a shift from descriptive to interventionist approaches. Until now, genetics has contributed mainly to understanding the natural history of autoimmune disorders, offering improvement in diagnosis and classification. Now it seems that the tools may be at hand to arrest the pathological process. One most promising lead is the deliberate generation of suppressor T cells *in vitro*, possibly followed by hybridisation with established cell lines and production of suppressor factors. Another possibility is to induce idiotype antibodies with restricted specificity for self-reactive immunocompetent cells. The coming years should provide several opportunities for designing specific immunotherapeutic manoeuvres rather than relying on nonspecific immunosuppression for the treatment of autoimmune diseases. □

The decline and fall of protein chemistry?

from Alan D. B. Malcolm

For more than 20 years the techniques of protein chemistry, including sequencing have played an enormous role in the development of biochemistry. It seems likely however that six papers in the *Journal of Biological Chemistry* (253, 5484; 1978) may be the zenith of this particular branch of science. These describe the complete sequence of β -galactosidase from *Escherichia coli*, which with a molecular weight of 116,349 is the largest protein so far sequenced. Its 1,021 amino acids gave rise to 72 chymotryptic peptides (*op. cit.* 5484), 24 cyanogen bromide peptides (*op. cit.* 5499) and 89 tryptic peptides (*J. biol. Chem.* 247, 5425; 1972) and the separation and ordering of these has taken A. Fowler, I. Zabin and colleagues at the University of California at Los Angeles eight years, and involved growing *E. coli* on [¹⁴C]lysine and [¹⁴C]arginine as well as [³H]methionine to increase the sensitivity of detection. The techniques of nucleic acid sequencing have developed so quickly that this is now much easier than protein sequencing—a fact which is acknowledged at the end of the last paper (*J. biol. Chem.* 253, 5521; 1978) where the authors state that the

sequence of the first 145 residues agrees with that predicted from the DNA sequence determined in W. Gilbert's laboratory at Harvard. It cannot be long before the rest of the DNA sequence is used to confirm or correct the protein sequence.

In this, the β -galactosidase sequencers have a problem similar to that of Ovchinnikov's group in Moscow who set out several years ago to sequence the subunits of RNA polymerase. They published the sequence of the α -subunit (molecular weight 36,512) last year (*FEBS Lett.* 76, 108; 1977) but before they could finish the two large subunits (β and β' , with molecular weights of 155,000 and 165,000) comes the news from Nomura's laboratory in Madison that part of the DNA is being sequenced thus making the protein sequence almost (but not quite) redundant. Nomura is using transducing phage λ *drif*¹⁸ which contains *rpoB* and *rpoC*—the adjacent genes which code for β and β' . Identification of precise start points for the protein on the DNA is made a lot easier by the fact that the N terminal tetrapeptide sequence has been known for some time (Fujiki & Zurek *FEBS Lett.* 55, 242; 1975) and it seems likely that the protein chemist will still have to provide such short sequences in the future.

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An interesting illustration of the changeover from protein to nucleic acid sequencing may be seen in the analysis of the phage λ repressor (Sauer & Anderregg *Biochemistry* **17**, 1092; 1978) where the authors have used a combination of the classical sequential Edman degradation, the more recent gas chromatography-mass spectrometry peptide sequencing and also the partial sequence of the *cl* gene (which codes for the repressor) obtained by the Maxam-Gilbert technique. The entire sequence of 236 residues was determined in the usual way and 179 of these were confirmed by GC-MS. The DNA sequence was then used both as further confirmation and also to resolve a disagreement with previous authors (Beyreuther & Gronenborn *Molec. gen. Genet.* **147**, 115; 1976) over the amino acids present at positions 14, 40 and 42. Like the *lac* repressor, the λ repressor binds to DNA through its N terminus and this region is highly basic with clusters of lysine and arginine at 3-5, 16, 17, 19 and 24-26.

Molecular analysis of the *lac* operon and its control regions is yet closer to completion with the recent publication of four papers (*Nature* **274**, 762; 1978) which include sequences for the promoter of the repressor gene (Calos *Nature op. cit.* 762) and the sequence of the repressor gene itself (Farabaugh *Nature op. cit.* 765). In both these papers the problem of obtaining enough DNA has been overcome by cloning restriction fragments shown to contain the relevant sequences in the plasmid vector pMB9, and again a transducing phage, λ h80d*lac*, which carries the *lac* operon, was useful as the original source of the DNA. The sequence of the repressor gene suggested that two peptides had been overlooked in the original sequence of the repressor (Beyreuther *et al. Proc. natn. Acad. Sci. U.S.A.* **70**, 3576; 1973), and also suggests that Gln should be Glu at positions 164 and 215. Beyreuther has checked the protein sequence (*Nature op. cit.* 767) and has found the missing residues—two are from a CNBr peptide at positions 231 and 232 and the other eleven at positions 148-158 are part of a tryptic peptide. Thus not only is DNA sequencing faster, but it is also less likely to lose fragments as a result of their small size or insolubility.

Of course it is still easier to purify a particular protein than to purify and characterise a particular segment of DNA but the latter is becoming easier all the time. In *Cell* (**14**, 673; 1978), Montgomery *et al.* report the isolation of the yeast cytochrome *c* gene. Several *EcoRI* restriction fragments of yeast DNA were found to hybridise to a synthetic oligodeoxynucleotide complementary to a region near the NH₂ terminus of the protein. A comparison

with the fragments obtained from a structural gene mutant where a G $\rightarrow$ T mutation abolishes an *EcoRI* site enabled the fragment containing the gene to be identified. The sequence of the first 91 nucleotides agrees exactly with the protein sequence. It will obviously not be long before phylogenetic trees based on nucleic acid sequences totally displace the less meaningful ones based on proteins.

Even the methods such as chemical modification developed to identify the residues vital for a protein's function now have a powerful rival in the technique of site-directed mutagenesis developed in Weissmann's laboratory in Zurich. This involves using a restriction enzyme in the presence of ethidium bromide to introduce a cut in only one strand of the DNA and then using the fact that DNA polymerase I will hydrolyse from the 5' end and re-synthesise at the 3' end of the break. By allowing this 'nick translation' to occur in the absence of some of the deoxynucleotide triphosphates and in the presence of a nucleotide analogue such as hydroxy dCTP (HODC), the analogue may be incorporated just once at a predetermined position. Since HODC can base pair with either G or A subsequent generations will either

be wild type or have a mutation at this site and the properties of the mutant may now be studied. So far this has been used to convert the QB phage coat protein cistron initiation sequence AUGGCA to AUAGCA or AUGACA or AUAACA and to study the effect on ribosome binding (Taniguchi & Weissmann *J. molec. Biol.* **118**, 533; 1978; *News and Views* **272**, 397; 1978). Perhaps surprisingly a G $\rightarrow$ A mutation in the fourth position actually increases the efficiency of ribosome binding whereas a G $\rightarrow$ A transition at the third position totally eliminates binding. The double mutant binds weakly. It seems as though the ability of the mutant A at position four to hydrogen bond to a U next to the CAU anticodon on tMet-tRNA must be responsible. Weissmann has now applied this trick to the gene for rabbit β -globin (albeit in a plasmid rather than in the animal) (Muller *et al. J. molec. Biol.* **124**, 343; 1978). Such an approach will clearly have a tremendous impact on attempts to establish structure-function relationships for proteins. Custom designed mutants seem likely to make much of today's elegant protein chemistry seem very crude. $\square$

Astronomers licked by QSOs and active galactic nuclei

from John Faulkner and Martin Gaskell

A RECENT workshop on quasars and active galactic nuclei hosted by Lick Observatory provided not only an appropriate topic for discussion at this centre of optical and theoretical work on emission line objects, but also provided a forum for the exchange of views (and occasionally insight) derived from other wavelength regions. Important contributions were made towards the resolution of long-standing problems; in other cases recently discovered problems became even more acute.

The subject is bedevilled for the non-specialist by the terminology used to describe the vast subclasses of objects observed (QSOs, BSOs, OSQs, BL Lacs, OVV's, NLRGs, BLRGs, AO 0235+164 objects and so on). It was not always clear that many of the participants bore the numerous fine observational distinctions between these objects in mind in their discussions, or even whether they should have. There did seem to be a tacit assumption that all QSOs and active galactic nuclei

(AGNs) are indeed manifestations of varying degrees of the same phenomenon.

The similarities and differences between radio-selected QSOs and optically-selected QSOs (OSQs) were discussed by several participants. Techniques for finding OSQs are improving. Less than 10% of these are turning out to be 'radio-loud'; and of the latter only a very small fraction are extended sources, which prompted D. Weedman's remark that "it's just ironic that radioastronomers found QSOs". A. Savage (Royal Observatory, Edinburgh) emphasised the problems of personal judgment in the selection of objects from prism plates. For example, one person might preferentially find high red-shifted, another low red-shifted OSQs on examining the same plates. It is therefore not yet possible to compare directly the surface densities of radio QSOs and OSQs. It does seem, however, that the surface density of OSQs increases to fainter magnitudes without any cutoff, in contrast to radio QSOs which have a peak in their magnitude distribution. Both Savage and P. Osmer (CTIO) remarked that

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radio-loud QSOs are far more likely to be optically variable than are radio-quiet QSOs.

No doubts at all were raised over the cosmological interpretation of emission line redshifts. Indeed almost the last nail in the non-cosmological redshift's coffin was hammered in by the acclaimed work of A. Stockton (Hawaii), who gave strong evidence for an association of galaxies with bright QSOs. Furthermore he showed that the redshift distributions of the associated galaxies were essentially symmetric about the emission line redshifts—thus lending great weight to the formerly disputed notion that the QSO emission line redshift is a 'true' cosmological redshift.

An important recent development in the often frustrated application of QSOs to cosmology has been the discovery and employment of the 'Baldwin effect' (a correlation between the CIV line strength and continuum luminosity; see Baldwin *et al.* *Nature* **273**, 431; 1978; *News and Views* **273**, 428; 1978). At the workshop it became increasingly clear that the Baldwin effect is most strongly seen in flat-radio spectrum QSOs, for which it was in fact first established. Osmer, for example, has now found faint weak-lined QSOs so that 'violation' of the effect by the radio-quiet QSOs can no longer be entirely attributed to an optical selection effect. In addition Harding E. Smith (University of California, San Diego) has identified a new subset of steep radio spectrum QSOs possessing unusually steep optical spectra (and of intrinsic interest in themselves) which do not satisfy the Baldwin effect. Nevertheless, C. M. Gaskell (Lick Observatory) reported that the Baldwin effect does seem to be holding up well in the face of new data for the flat radio spectrum objects. However, the use of the relationship to derive a value for the deceleration parameter q_0 of well over 0.5 provoked perhaps the most lively controversy of the workshop. Some support for a q_0 of around 1 came from R. F. Green (Caltech) in his application of the Baldwin effect to satellite ultraviolet observations of two flat radio spectrum QSOs. This holds promise, as further data comes in from the International Ultraviolet Explorer (IUE), that a wider redshift range can be used and applied to cosmology. Naturally, for that application it will be important to demonstrate that evolutionary effects are either absent or can be correctly allowed for. In this respect the infrared observations of Puetter (University of California, San Diego) and the IUE observations of J. B. Oke (Caltech) and Green were important for showing the similarities between high and low redshift QSOs when regions of the same rest wave-

length are compared.

All these observations accentuated recent problems in understanding the relative intensities of the hydrogen emission lines in both QSOs and Seyfert galaxies. Problems which had arisen earlier on a comparison of lines in composite spectra (which might therefore be suspect if QSOs of differing redshifts were dissimilar) were brought into stark focus now as it became clear that the Ly α to H β line ratios differed from those predicted by recombination theory by a factor of ten, and that this could not be explained by simple external reddening alone. Despite the endeavours of H. Netzer (Texas) and R. London (JILA, Colorado), no single mechanism seemed able to produce the desired suppression of Ly α or enhancement of the Balmer lines.

The nature of QSO absorption lines (that is, whether they are due to intrinsic or intervening matter) remains unresolved. There are still a large number of unidentified lines, differing from QSO to QSO. A. E. Wright (A.A.O.) made the (serious) suggestion that progress might be achieved by assuming that all absorption lines blueward of Ly α emission which had formerly been assumed to be all Lyman lines, should instead be assumed not to be Lyman lines at all. A. Wolfe (Pittsburgh) reported the detection of a third 21-cm absorption line QSO.

G. K. Miley (Leiden) in collaboration with J. S. Miller (Lick), produced the first significant correlations between the optical spectra and radio morphology of QSOs. Extended sources have broader and more complex H β profiles. A direct outcome of the workshop was a collaboration between Miley and R. Angel (Arizona) which showed a striking correlation between the position angles of the optical polarisation and the large scale radio structure of the double sources.

No startlingly new theoretical ideas were produced, although many old scenarios have been pushed much further. In particular the detailed evolution of the dense nuclei of galaxies has been pursued by M. C. Begelman (Cambridge) and S. I. Blinnikov (Moscow). It seemed that all routes led to black holes.

The organisers had assigned M. J. Rees (Cambridge) the heroic task of chairing the session on complete models; a job which proved even more challenging when none was forthcoming. The model of R. D. Blandford (Caltech) and Rees, which tried to explain BL Lacertae objects as ordinary QSOs viewed with their highly relativistic jets pointing at us, met with firm opposition from the observers on whom these jets were supposedly aligned. □



A hundred years ago

The Sea-Serpent Explained

ON Monday, August 5, a number of geologists crossed in the Folkestone boat to Boulogne, to study the interesting formations of that neighbourhood, and, when about three or four miles from the French coast, one of these gentlemen suddenly exclaimed, "Look at that extraordinary object passing across the bow of the steamer, about a mile or a mile and a-half in advance of us!" On turning in this direction there was seen an immense serpent, apparently about a furlong in length, rushing furiously along at the rate of fifteen or twenty miles an hour; it was blackish in front and paler behind; its elongated body was fairly on the surface of the water, and it progressed with an undulating or quivering motion: *mirum erat spectaculum sane*.

Of course many suppositions were immediately started to account for this extraordinary phenomenon, but they quickly changed and settled into the fixed idea that the object before them could be nothing less than the great sea-serpent himself; for,—

"Prone on the flood, extended long and large,
Lay floating, many a rood, in bulk as huge
As whom the fables name of monstrous size,
Leviathan; which, God of all his works
Created hugest, that swim the ocean stream."

The writer fortunately had with him one of Baker's best opera-glasses, and, after a few moments' use of this little instrument, the wonder was satisfactorily resolved. The first half of the monster was dark and glittering and the remainder of fainter hue, gradually fading towards the tail. The glass did not determine the matter until the extreme end was reached, and then it was seen to consist of a mass of birds in rapid motion; those that were strong on the wing were able to keep well up with the leaders, and so make the head appear thicker and darker by their numbers, whilst those that had not such power of flight were compelled to settle into places nearer and nearer the tail. Doubtless these birds were shags (*Pelicanus cristatus*) returning to their homes for the night from the distant waters in which they had been fishing, during the day; perchance it may be wrong to assert positively as to the variety of bird, but inasmuch as the writer has often seen shags on the Cornish coast in smaller numbers returning in single or double file to their roosting places, and since it is stated in works of natural history that they have been noticed occasionally flying in this peculiar manner to the number of a thousand or more, it does not appear an unwarranted liberty in supposing that they really were *Pelicanus cristatus*.

From *Nature* **18**, 5 Sept., 489; 1878.

review article

Prospects for the use of amorphous semiconductors in solar energy conversion

J. I. B. Wilson & D. Weaire*

There is increasing interest in amorphous silicon as a low cost material for photovoltaic solar cells. Its possible uses extend to other methods of solar energy collection such as photothermal and photogalvanic conversion.

THE mutually stimulating but unpredictable interactions of pure and applied science are well exemplified by the history of amorphous semiconductors. These materials emerged from obscurity in the late 1960s, when new electronic switching devices, based on amorphous chalcogenides, excited widespread interest¹. Of course, this was not the first application of amorphous solids. They already had many uses, both mundane (for example, common glasses) and sophisticated (for example, amorphous Se, in xerography). Nevertheless, the inspiration for much of the concentrated academic activity in this area during the 1970s lay in the prospect of a new sub-field of electronics ('Ovonics') where the established picture of a crystalline semiconductor might not be at all relevant. Important new concepts have emerged, such as that of Anderson localisation², but the theory of amorphous semiconductors remains a difficult subject, rarely united happily with experiment³.

Small wonder then, that many have preferred not to jump in at the deep end by exploring the switching effect in the multi-component glasses. There have been obvious attractions in the study of the elemental amorphous semiconductors, particularly Si and Ge. It was, therefore, chiefly in the context of these comparatively simple systems that the debate between the rival microcrystalline and random network models of microscopic structure was conducted⁴. The consensus now favours the latter as the better conceptual basis for the description of amorphous Si and Ge.

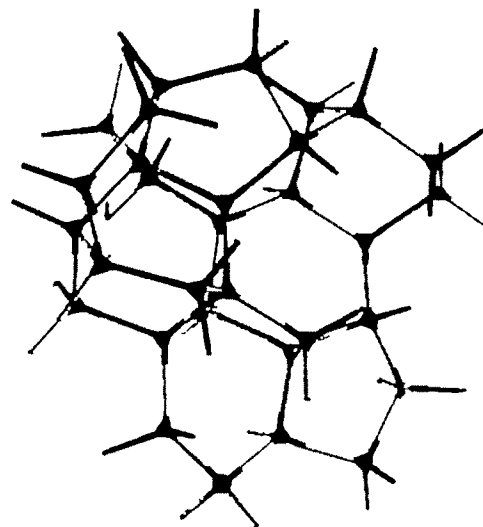
There have been no important applications of amorphous Si and Ge (apart perhaps from optical coatings, where a wide variety of materials find a use). However, it has recently been shown that amorphous Si, when suitably prepared, can be used to make conventional semiconductor devices⁵. This was first demonstrated using the r.f. glow discharge decomposition of silane to deposit the amorphous film and varying the doping by the admixture of gases containing p-type and n-type dopant atoms⁵. It was then realised that the incorporation of hydrogen which is incidental in this process is essential for its success. Various other methods (such as sputtering⁶), suitably adapted to incorporate a small proportion of hydrogen, are under investigation. It remains to be seen whether the glow discharge process will be preferred when these alternatives have been fully explored.

The role of hydrogen is to reduce the density of electronic states in the gap region (typically from $10^{20} \text{ cm}^{-3} \text{ eV}^{-1}$ to $10^{18} \text{ cm}^{-3} \text{ eV}^{-1}$). These states are presumably associated with 'dangling bonds' in hydrogen-free samples, to which the hydrogen atoms are bonded when present. Figures 1 and 2 give an impression of the general nature of this material—a random network in which some of the Si atoms have one or more H atoms bonded to them.

In this article, we review some potential applications of amorphous Si in the field of solar energy conversion. These are not confined to the junction devices mentioned above, that is, solar cells, but these certainly constitute the most promising prospect.

How can such an intrinsically messy material have advantages over crystalline Si, on which so much of our device technology is already based? The answer lies in the anticipation of a comparatively low cost for the industrial production of amorphous Si in thin films of large area. In this regard, solar energy research runs

Fig. 1 Fragment of a random network model of amorphous silicon, in which tetrahedrally coordinated silicon atoms form an infinite non-periodic structure.



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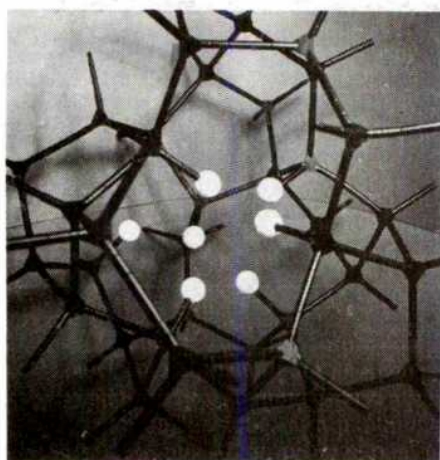


Fig. 2 Fragment of a random network model of hydrogenated amorphous silicon, with hydrogen atoms bonded to some silicon atoms.

counter to the trend of modern electronic device engineering in which 'small is beautiful'. The question then is whether acceptable efficiency and reliability can be obtained with an amorphous cell. This is still not clear but sceptics should be reminded that, had the single crystal window been invented first, they might be equally pessimistic about that familiar use of glass.

Photovoltaic conversion of solar energy

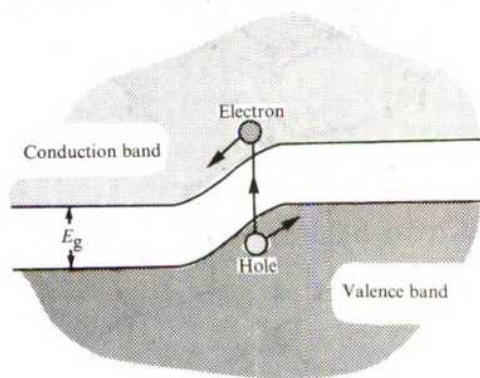
Photovoltaic cells, which convert light into electricity by a purely electronic process (that is, without chemical change) have been used for some years to power satellites. Indeed most of the development of high efficiency Si cells in the 1950s and 1960s derived from the needs of the space industry. They have also provided power in terrestrial sites where their convenience and reliability outweighs their high capital cost. Such applications will remain very limited until a much cheaper cell is developed. New research programmes in the US⁷ and Europe⁸ are aimed at this cost reduction.

All photovoltaic cells exploit the fact that a semiconductor will absorb radiation of sufficiently high frequency to excite an electron across the band gap, of magnitude E_g , and that electrons excited in this way return to the valence band ('recombine') only after comparatively long times (10–100 μ s).

In a p–n junction the energy levels associated with the valence and conduction bands vary in the manner idealised in Fig. 3, dropping by an amount E_g across the junction. Photogenerated electrons will move to the left (while the 'holes' left behind in the valence band move the other way).

If the junction is isolated (open circuit condition), the resulting charge imbalance will set up a potential difference V_{oc}

Fig. 3 Electronic energy levels at an idealised p–n junction. An electron–hole pair created by absorption of radiation near the junction is separated as shown (photovoltaic effect).



opposing the charge transfer. The system can only come into equilibrium when this exactly balances the change in electron energy levels at the junction, that is $eV_{oc} = E_g$ in our simplified picture.

This is the essence of the 'photovoltaic effect'. If the junction is incorporated in a circuit, a current I will flow and V will be correspondingly reduced. The power output is then simply IV and this is divided (for the optimum load) by the solar energy flux to give the maximum efficiency of the cell.

A narrow gap semiconductor absorbs most of the solar spectrum but wastes much of it as electrons are excited well above the gap and their surplus energy is merely converted into heat. On the other hand, a wide gap semiconductor fails to absorb at the infrared end of the spectrum. Thus the continuous frequency distribution of solar radiation (Fig. 4) enforces a compromise, favouring the choice of a semiconductor of band gap in the region of 1.5 eV. Even for this, the power efficiency is reduced to <50% by the waste at the low and high ends of the spectrum. Realistic models of junction behaviour¹⁰ reduce the theoretical efficiency to ~25% and practical considerations (grid contacts, and so on) reduce this figure still further. Most commercial Si cells achieve ~10–15%. The best laboratory result is 23% for GaAs cells in concentrated sunlight.

Schottky barrier solar cells are based on a metal–semiconductor junction (sometimes modified by the inclusion of a thin insulating layer at the junction). The essential structure of both types of cell is shown in Fig. 5.

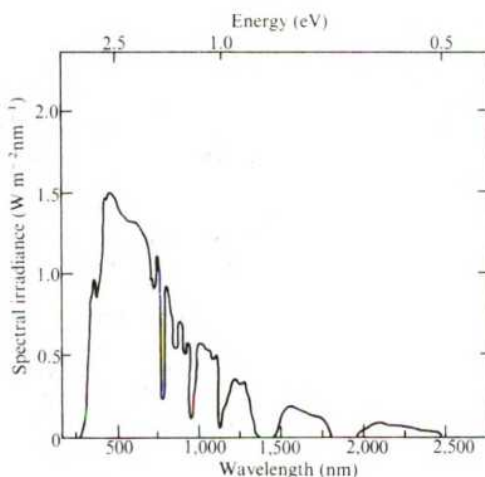


Fig. 4 Spectrum of incident solar radiation at the Earth's surface, for normal incidence (AM1).

Commercially available cells at present use semiconductor wafers sliced from expensive single crystal boules. Two routes are being taken to reduce the cost.

First, the addition of lenses or mirrors to an expensive cell may produce a cheaper module by concentrating sunlight upon the cell. This approach is most appropriate when a large proportion of insolation is direct. It is of particular value in association with GaAs cells, which are efficient solar energy converters at higher operating temperatures than silicon.

Second, silicon for solar cells does not require such perfection and stringent purity control as for most other semiconductor devices and so polycrystalline boules or extruded ribbons may be of adequate quality. Taken to its logical extreme, this approach leads to the consideration of polycrystalline or amorphous thin films.

On the whole, polycrystalline thin-film solar cells, using a variety of semiconductors, have been disappointing. Often only fine grained polycrystalline films can be grown with unsatisfactory electrical behaviour due to grain boundaries. In other cases, it has been difficult to grow uniform defect-free films more than a few mm^2 in area, and sometimes the cells have shown excessive degradation after a short period of operation.

Amorphous silicon avoids some of these problems and even has certain marginal advantages over crystalline silicon. For instance, it has a bandgap 1.5–1.7 eV, closer to the optimum than that of crystalline silicon (1.1 eV), and furthermore absorbs radiation more strongly just above the gap. Radiation hardness is sometimes mentioned (particularly in the context of satellite power stations). However, while it may be true that "one of the unique properties of amorphous semiconductors is their low sensitivity to high energy radiation..."¹¹ there is relatively little in the published literature relating to this topic.

Current laboratory work is concentrated on the achievement of acceptable efficiencies. 'Acceptable' is generally taken to mean at least 8% but depends strongly on the application and its location.

Within a short space of time various p-n and Schottky cells based on amorphous Si have been reported with efficiencies of the order of 5%, as summarised in Table 1. Theoretical estimates of the limiting efficiency of such cells have ranged from 8 to 15%, and it seems reasonable to expect that the lower figure can be attained in the near future. The factor which is most significant in reducing the efficiency in comparison with single crystal cells is the short diffusion length of the charge carriers.

Table 1 Performance of amorphous silicon solar cells, under AM1 or similar conditions

Cell structure	Open circuit voltage (mV)	Short circuit current density (mA cm^{-2})	Efficiency (%)	Ref.
p-i-n	575	10.5	2.4	12
Pt-n	800	12	5.5	13
Ni-TiO _x -n	680	13	4.8	14

Only electrons and holes excited within about $1 \mu\text{m}$ of the junction survive to contribute to the photogenerated current. In a crystalline semiconductor the active layer may be as much as 0.5 mm thick. It remains to be seen whether this drawback is unavoidably inherent in the amorphous structure of the material or can be mitigated by refined preparative methods. However, little has been done (or, at least, published) on the optimisation of the doping profile close to the junction, which may yield some improvement.

It should be stressed that the results quoted above are for rather small area cells ($\approx 1 \text{ mm}^2$). It is a far cry from a laboratory cell of a few mm^2 to the orbiting solar power satellite suggested by Glaser¹⁵, with solar cells totalling 50 km^2 .

Photogalvanic and photochemical cells

Figure 6 shows the essential features of a photogalvanic cell. Again electrons are excited across the gap of a semiconductor at one electrode. Unfortunately most semiconductors with a suitable band gap are chemically unstable in electrolytes. TiO₂, a chemically stable compound which has featured in many studies, has a rather wide gap ($\approx 3 \text{ eV}$). Photochemical cells, in which TiO₂ has also been used; produce chemical fuels such as hydrogen from water. Promising results have recently been obtained with CdSe (ref. 16) ($E_g \approx 1.7 \text{ eV}$).

Amorphous semiconductors may offer new possibilities for such cells. Favourable factors are their ability to form strongly adherent thin films on a variety of substrates and low cost, which in this case might make regeneration economically feasible.

Photothermal conversion

Flat-plate solar collectors for heating water are installed in thousands around the world. These operate by the 'greenhouse effect', which is generally taken to mean that the selective transmittance of glass is the dominant feature. Most of the solar

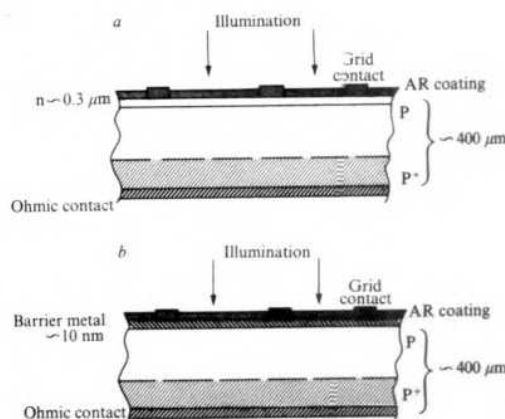


Fig. 5 Schematic structure of: *a*, conventional n-on-p silicon solar cell; *b*, metal-semiconductor (Schottky) junction cell. The dimensions are typical of crystalline solar cells.

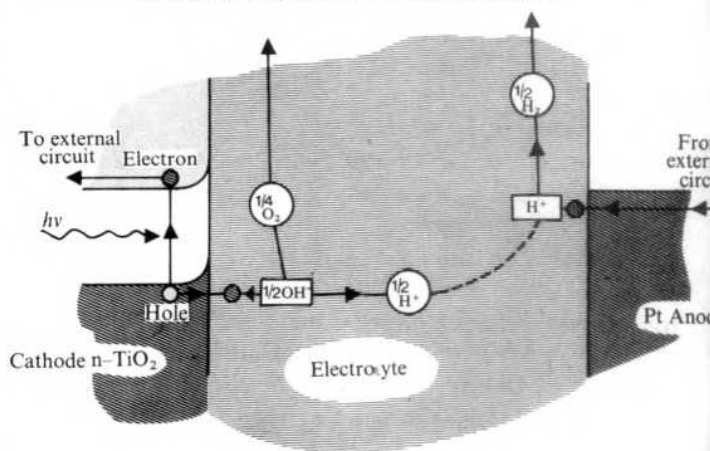
radiation passes through the glass but outgoing thermal infrared radiation, emitted by hot surfaces within the glass enclosure, is blocked. Consequently the temperature reached by an absorber plate is greater when a glass cover is in place. In practice the glass cover is equally important in reducing energy loss by convection, conduction, and evaporation, unless the temperature within is very high.

The cut-off point for transmission through glass lies around $3 \mu\text{m}$, so some solar radiation is excluded from the enclosure, but a longer wavelength cut-off would mean that more energy would be lost by radiation. A compromise is to make the cut-off as sharp as possible, at about $2 \mu\text{m}$. Although thin-film coatings can be applied to the glass to bring this about, there has been more interest in selective coatings for the absorber plate, for which the potential gain in collected energy is greater. A selective absorber will absorb visible radiation efficiently, but will not absorb infrared radiation. This means that equally it will emit visible radiation but not infrared.

Practical selective surfaces are generally semiconducting compounds, although their preparation may mean that their composition is obscure. Proprietary coatings include various Ni/Cr oxides/sulphides, which can have solar absorptances of up to 0.98 and infrared emittances of less than 0.2, even at the elevated operating temperatures.

Single-layer films such as these utilise the high infrared reflectance of a metal substrate (and consequently low infrared emittance) whilst providing an efficient absorber of visible radiation. It is only with multi-layer coatings that a sharp cut-off can be achieved, but this increases the complexity and cost of the

Fig. 6 Photogalvanic cell. An electron is removed from the electrolyte at the photo-cathode and one is injected into the electrolyte at the anode. Oxygen and hydrogen are evolved in this cell, by the simplified redox reactions shown.



collector. Semiconductors with a suitable bandgap for this application tend to have high refractive indices and are, therefore, a bad match to the low refractive index of air unless an anti-reflection coating is added. Sometimes it is possible to reduce the refractive index of a film by preparing it with a large density of minute voids.

The two drawbacks preventing the use of selective coatings on all flat-plate collectors are (1) their cost, and (2) their degradation at high operating temperatures. Degradation of absorber coatings may be caused by thermal expansion mismatch between film and substrate, leading to flexing, or by thermal migration of atoms between the film(s) and substrate.

Now, amorphous silicon produced by r.f. glow discharge decomposition of silane adheres very strongly to both metallic and ceramic substrates, because the plasma is an effective surface scourer. However its band gap is rather far from the optimum absorption cut-off of ~ 0.6 eV ($2\ \mu\text{m}$). Amorphous silicon films prepared by electron-beam evaporation in a vacuum chamber have such a high density of defect states within the bandgap that they will absorb energies further into the infrared. On balance it seems preferable to use glow discharge amorphous silicon, which can be made with a low free-carrier density (high electrical resistivity) and low 'gap states' density, and so use its low infrared emittance. Note, however, that amorphous semiconductors may be alloyed comparatively easily to tailor their optical properties. In this case, the solar absorptance of amorphous silicon may be improved by alloying it with boron¹⁷. It is expected that films of $\text{Si}_{1-x}\text{B}_x$ prepared by the glow discharge decomposition of SiH_4 and B_2H_6 may yield solar absorptances of up to 0.91 and infrared emittances of <0.09 even at the elevated temperatures reached by solar collectors.

At temperatures above $\sim 400^\circ\text{C}$, amorphous silicon may begin to recrystallise with some loss of the desired properties.

Significant changes should only occur after many years of collector use at this temperature. A second possible problem is that at these temperatures (which may be reached by concentrator collectors) hydrogen may be evolved from the film. Hydrogen evolution tends to decrease the bandgap¹⁸, which is not necessarily undesirable. Annealing of amorphous silicon films (that is prolonged periods at high temperatures) also seems to reduce the intensity of infrared absorption bands near $5\ \mu\text{m}$ (which are due to Si-H stretch modes), and so should reduce infrared emittance. This may prove to be a situation in which ageing is beneficial.

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articles

Characterisation of dielectric loss in solids and liquids

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The measured dielectric properties of a range of solids and liquids, as a function of frequency, have been examined and the loss has been shown to fit a generalised empirical relationship. This includes the Cole-Cole, Fuoss-Kirkwood, Cole-Davidson and Williams-Watts functions as particular cases and implies that a more generalised approach to the dielectric properties of materials is required than that given by the conventional distribution function extension of Debye's method, or by the dipole autocorrelation function of Williams and Watts.

OUR present understanding of the dielectric properties of materials is based on the Debye theory of a relaxing dipole interacting with an applied electric field¹. Early work showed that a set of exactly equivalent, non-interacting, dipoles characterised by a single relaxation time adequately explained the behaviour of weak dipolar solutions or dipolar molecules in the gas phase, but was insufficient to account for the broader frequency range over which dispersion was observed in solids and liquids in the frequency range below $\sim 10^{10}$ Hz. This difficulty was circumvented by the consideration of distributions of relaxation times² or by dipole autocorrelation³ in these more interactive media. These approaches have led to the situation where experimental measurements are analysed in terms of the

degree of fit to Cole–Cole, Cole–Davidson, Fuoss–Kirkwood, Williams–Watts or other empirical functions.

The results of a critical examination of the frequency dependence of the permittivity in a number of dielectric materials are reported here and compared with these standard functions. It is shown that the overall pattern of behaviour is broader than can be characterised by any one particular function and an empirical, but quite general, dielectric loss relationship is proposed and used to characterise the observed behaviour. The analytical method is based on the technique of temperature reduced variables⁴ with both logarithmic permittivity and frequency. The latter is common but the former is equally applicable and Jonscher⁵ has already shown that for frequencies greater than

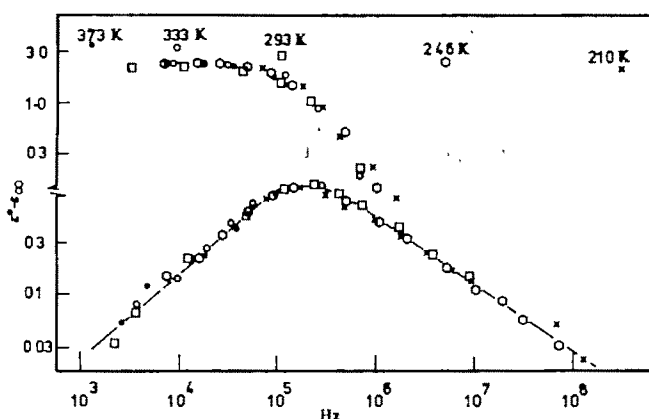


Fig. 1 The real and imaginary components of the permittivity of pentachlorotoluene⁷ as a function of frequency. The diagram has been plotted in the form of a temperature reduced master curve to show the power law characteristics at frequencies below and above that of the peak loss. The scales are correct for $T = 293$ K and the curve shifts are given by the temperature labelled datum points at the top of the diagram. The curve through the loss characteristic is given by equation (1) with the parameters listed in Table 1.

the loss peak value, ω_p , both the real and imaginary components of the complex permittivity are proportional to $\omega^{-(1-n)}$, where ω is the frequency of measurement and n lies in the range between zero and unity.

By forming a master curve from the data over suitable temperature ranges the accuracy with which the pattern of permittivity behaviour, as a function of frequency, could be determined has been increased. In all cases clear evidence of power law behaviour with ϵ'' proportional to $\omega^{-(1-n)}$ for $\omega > \omega_p$ and proportional to ω^m for $\omega < \omega_p$ has been observed, a general relationship that is masked in the more common linear permittivity/logarithmic frequency plots⁶. Several empirical functions with these power relationships as limiting cases, were investigated and it has been found that the relationship

$$\epsilon'' \propto \frac{\omega^m}{(\omega_p^{2s} + \omega^{2s})^{(m+1-n)/2s}} \quad (1)$$

gave remarkably good agreement with the experimentally determined plots. The parameter s dominates in the region of the peak only, that is $\omega \approx \omega_p$, and determines the curvature between the decaying power characteristics, low values of s giving a broad, flat, peak in the loss. The parameters m , n and s all lie in the range between zero and unity.

Equation (1) obviously includes Jonscher's relationship at high frequencies. For the particular case of $m = (1-n) = s = 1.0$ equation (1) degenerates to the Debye case, and with $m = (1-n) \neq 1.0$ Cole–Cole and Fuoss–Kirkwood plots can be derived, as can the Cole–Davidson characteristic when $m = 1.0$ and in this case $\beta_{CD} = (1-n)$. The Williams–Watts plots of ϵ'' as a function of frequency³ also show the power characteristics but

in this case there is no simple relationship between β_{WW} and either of the gradients m and $(1-n)$ although increasing values of β_{WW} gives increasing values of both gradients.

Examination of experimental data

Published loss data for a range of dielectric materials, each over a set of temperatures, was plotted logarithmically on both permittivity and frequency axes. A single master curve was then constructed by pure translation, in both axes, for each temperature. The choice of axial shift was arbitrary, but minimal scatter in the reduced data points was sought. In a few cases both the real and the imaginary components of the permittivity were available and, as the translation is identical for each of these, this made the choice of axial shift more certain. Along with the experimental points a datum from the original plot was marked to give the magnitude of the translations. Note that the tracing technique gives the inverse of the temperature dependence for both the permittivity and frequency. The final master curve

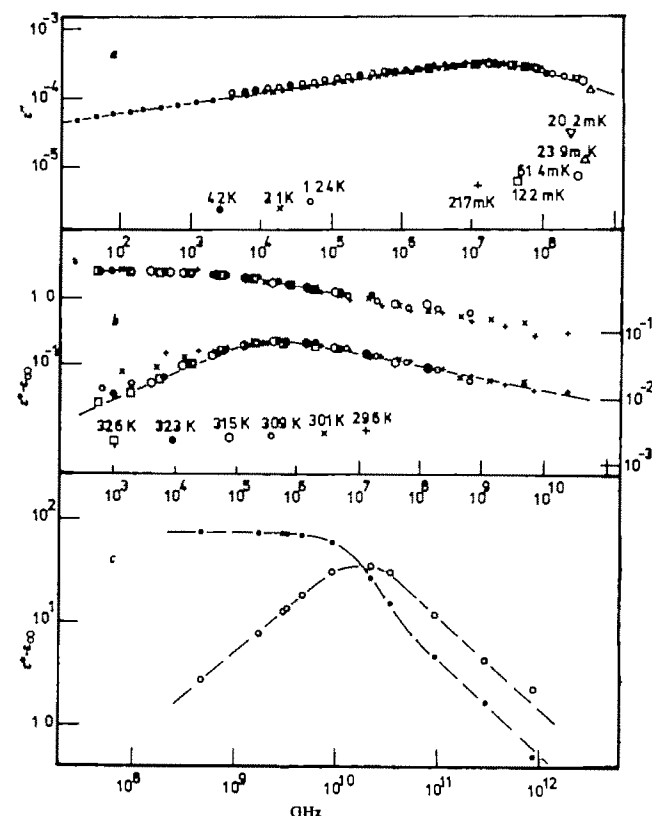


Fig. 2 Temperature reduced plots of the permittivity of *a*, suprasil glass (J. le G. Gilchrist, personal communication) (scaled at 4.2 K); *b*, poly- γ -benzyl-L-glutamate⁸ (scaled at 326 K); *c*, water at 293 K (ref. 9). In all cases the curves through the loss peaks are given by equation (1) with the parameters listed in Table 1. The suprasil glass shows the frequency extent of the low frequency power characteristic. In (*b*) the dotted curve through the real part of the permittivity is a Kramers–Kronig transformation of the loss characteristic. The measured gradients of the loss characteristics for water are 0.963 and -0.968 indicative of a Cole–Cole process, but the specimen is clearly not Debye-like as the real component of the permittivity does not have a slope of -2.0 but obeys the Jonscher relationship in being parallel to the loss at frequencies greater than that of the loss peak.

contains only the information that was originally available but presented in a form that is more easily analysed, as the superimposition of a number of equivalent measurements reduces the experimental inaccuracies. In all cases the master plot showed a characteristic loss peak with linear wings, clearly indicative of the power law characteristics exhibited by equation

(1). The gradients m and $-(1-n)$ were determined and the parameter s chosen to give a good fit of the empirical relationship to the master curve in the region of the loss peak. In a few cases, where the real part of the permittivity was available, a Kramers-Kronig transform of the loss relationship, equation (1), was computed and excellent agreement with the experimental data was obtained for the real part of the permittivity.

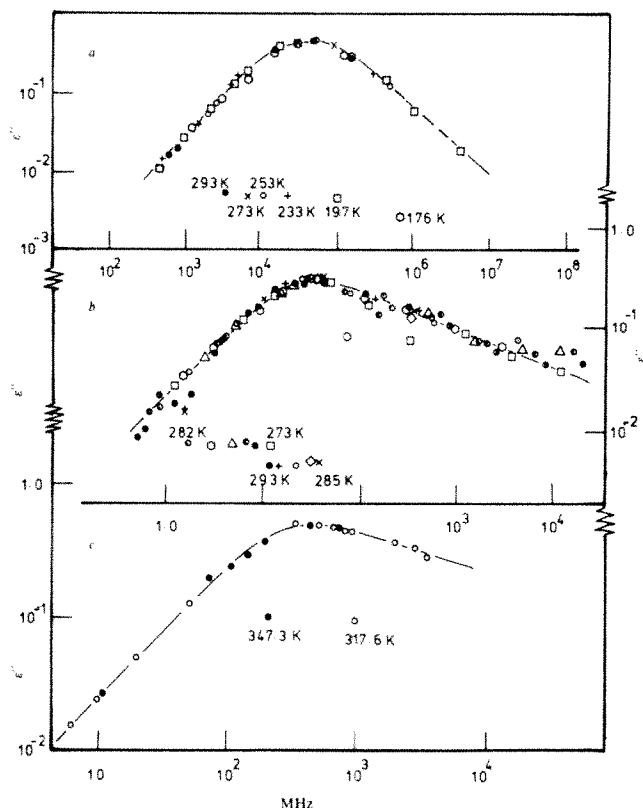


Fig. 3 Temperature reduced plots of the permittivity of *a*, 2,4,6-tri-*tert*-butylphenol¹² (scaled at 293 K); *b*, butyl stearate¹⁴ (scaled at 273 K); *c*, liquid menthol¹³ (scaled at 317.6 K). All three specimens exhibit a low frequency slope of 1.0 and the curves through the data points are given by equation (1) with the parameters listed in Table 1. This behaviour is equivalent to a Cole-Davidson distribution of relaxation times.

Figure 1 shows the reduced plot for both the real and imaginary relative permittivity for pentachlorotoluene from the data given by Turney⁷. Jonscher's power relationship for frequencies greater than the peak with $(1-n)=0.64$ is clearly shown in the loss curve but the real component has merged with the infinite frequency value and could not be determined with any accuracy. The low frequency power relationship, with $m=0.87$ is well defined and the curve through the loss data points is the relevant plot of equation (1) with s equal to unity.

The extent of the low frequency relationship is shown by Gilchrist's measurements on Suprasil glass at very low temperatures (personal communication), Fig. 2*a*. In the reduced plot, six decades of frequency exhibit the characteristic, and again the degenerate form of equation (1), $s=1.0$, applies. The fully developed proportionality between the real and imaginary parts of the permittivity for poly- γ -benzyl-L-glutamate⁸ is shown in Fig. 2*b*. In this plot, as in Fig. 1, the real and imaginary components have been displaced arbitrarily on the permittivity axis for clarity. The ratio of the absolute values of the permittivities at high, reduced, frequencies shows the predicted $\tan[(1-n)\pi/2]$ relationship⁵. At low temperatures the low, reduced, frequency data show deviation from the power characteristic due to an increasing distortion from a second, lower

Table 1 Dielectric properties

Material	m	$1-n$	s	Plot no.	Refs
Pentachlorotoluene	0.87	0.64	1.0	1	7
Suprasil glass, $T \leq 4.2$ K	0.15	0.31	1.0	2	*
Poly- γ -benzyl-L-glutamate	0.42	0.19	0.9	3	8
Water at 293 K	0.96(3)	0.96(8)	1.0	4	9
2:4:6 Tri- <i>tert</i> -butylphenol	1.0	0.66	1.0	5	12
Menthol (liquid)	1.0	0.22	1.0	6	13
Butyl stearate, $f > 10^9$ Hz	1.0	0.34	1.0	7	14

* J. le G. Gilchrist, personal communication

frequency, loss peak, the frequency separation between the two peaks decreasing as the temperature is lowered. The best fit master curve for the loss data required that s should be 0.9 ± 0.03 . The other parameters are listed in Table 1. In Fig. 2*b* as in Fig. 1, the temperature data lie on a straight line showing that there is a power relationship between the temperature dependence of the frequency shift, which is included in the term ω_p , the peak frequency, and the temperature dependence of the magnitude of the permittivity.

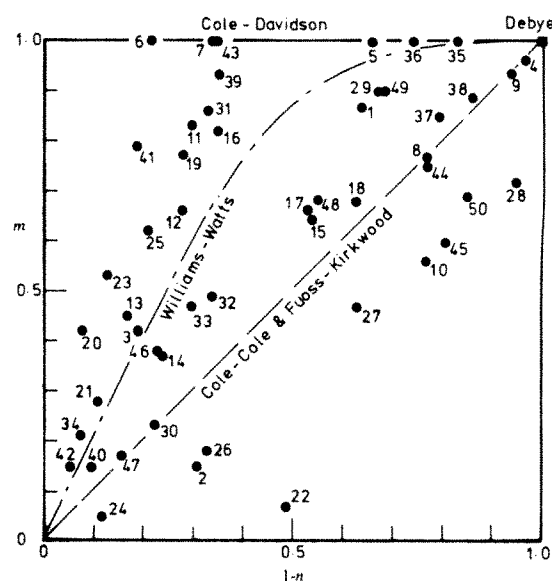


Fig. 4 A plot of the gradients m and $(1-n)$ determined for 50 loss peaks covering a wide range of polymeric and non-polymeric solids and liquids. Superimposed on the plot are the regions of validity of the Cole-Cole, Cole-Davidson, Fuoss-Kirkwood and Williams-Watts functions. Tables 1 and 2 list the gradient values.

None of these three plots show Debye, Cole-Cole or Cole-Davidson behaviour. The closest that has been observed to either Debye or Cole-Cole are the selected values of Mason *et al.*⁹ for water at 20 °C, which are presented in Fig. 2*c*. The deviation from equality in the magnitudes of the slopes, $m=0.963$, $(1-n)=0.968$, is within the accuracy of the determination of the slopes. The figure, however, shows a clear divergence from Debye behaviour in that the Jonscher relationship at high frequencies excludes the ω^{-2} decrease in the real part of the permittivity. Only if the loss decays exactly as the inverse of the frequency can the square law decrease be observed. The line through the loss data is, again, the empirical relationship. Ice at 181 K (ref. 10) and solid D₂O (ref. 11) have been observed to show similar equality in the gradients in log/log master plots and their characteristic parameters are given in Table 2.

Table 2 Master plots and characteristics

Material	Plot no.	Refs
H ₂ O at 181 K	8	10
D ₂ O, $T \leq 262.3$ K	9	11
Butyl stearate, $f < 10^9$ Hz	10	14
Poly- <i>n</i> -butyl methacrylate	11	15
Polymethyl methacrylate	12	6
Polymethyl methacrylate	13	15
Polypropylene	14	16
Amorphous polyacetaldehyde	15	17
Polyethylene, $4.2 \leq T < 18$ K	16	*
Oxidised high density polyethylene, $T < 4.2$ K	17	*
Deuterated high density polyethylene, $T < 4.2$ K	18	*
Polyethylene terephthalate		
α	19	18
β	20	18
Polyvinyl fluoride	21	19
Polyvinylidene fluoride		
α	22	20
β	23	20
γ	24	20
Polydian carbonate	25	21
Polyacronitrile	26	22
<i>n</i> -docosyl bromide	27	23
Neo-hexanol	28	24
5 methyl—3 heptanol		
α	29	25
β	30	25
3 methyl—3 heptanol		
α	31	25
β	32	25
Cyclohexyl chloride in polystyrene		
α	33	26
β	34	26
Tricyclohexyl carbinol, $f > 10^9$ Hz	35	27
$f < 10^9$ Hz	36	27
Picric acid	37	28
Pinacol hydrate	38	29
Chlorobenzene-pyridine, 43.4%		
α	39	30
β	40	30
Chlorobenzene- <i>cis</i> -decalin,		
α	41	25
β	42	25
Benzene, $f > 10^9$ Hz	43	31
<i>P</i> -methoxyphenylazoxy- <i>p</i> '-butylbenzene, nematic phase	44	32
isotropic phase	45	32
Polychloroprene		
α	46	33
β	47	33
Ba Ti ₄ MgO ₁₆	48	34
AgNa(NO ₂) ₂	49	35
Natural quartz containing impurity ions	50	36

* J. le G. Gilchrist, personal communication

Cole–Davidson behaviour, $m = 1.0$, has been observed in a number of materials, particularly when the loss peak has been observed in the frequency range from 10^7 to 10^{10} Hz. Figure 3 shows three sets of data with this characteristic, all of them from this frequency range, and all of them showing agreement with the empirical relationship. The characteristic parameters of these, and several other materials, are given in Table 1.

Some 40 materials have been examined using the technique described above and as some of these showed both α and β loss peaks about 50 loss characteristics have been determined. Figure 4 summarises the accumulated data in the form of a plot of m against $(1 - n)$. For clarity the points have been labelled and Tables 1 and 2 give a key to the data. It is apparent that the

distribution is not random but tends to cluster above the diagonal line $m = (1 - n)$. As well as the characteristic points the plot also shows the region of validity of the standard distribution and autocorrelation functions. The Debye characteristic is a single point, and is indicated by a square at (1, 1). The Cole–Davidson function requires that m should be unity and hence is given by the line along the top of Fig. 4, whereas the symmetry of the Cole–Cole and Fuoss–Kirkwood functions requires that data exhibiting this characteristic should lie on the diagonal from (0, 0) to (1, 1). The Williams–Watts expression is more complex, exhibiting a gradient of two for small values of β_{ww} and then smoothly merging with the upper limit for larger values of β_{ww} . The plot shows that although a small number of loss peaks lie on these characteristics the general distribution is much broader than these specific cases and that a more general approach to the nature of dielectric loss is required.

Conclusions

Our understanding of the dielectric behaviour in solids and liquids based on specific distributions of relaxation times or autocorrelation functions seems to be unsatisfactory. The force-fitting of experimental data into two or three types of behaviour is physically unfounded in view of the information presented in Fig. 4. The empirical loss characteristic proposed, equation (1), does not, at present, yield any physical understanding of the processes involved but has been shown to describe the experimental observations satisfactorily. The necessity for three parameters is clear as the loss curves are characterised by two power laws and a region of variable curvature. An empirical distribution function containing m , $(1 - n)$ and s could be generated but would contain no more understanding of the processes leading to loss than the empirical loss function itself. Rather, the function can be used as a test for new, more generalised, theories. One approach resulting in an expression for dielectric loss of the form of equation (1) will be presented in a later paper. Correlation of the parameters m , $(1 - n)$ and s , and of the temperature dependence of the frequency and permittivity datum shifts with the physical properties of the solid and liquid materials should begin to yield valid information on the precise nature of the interaction of electric fields and real materials.

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Bedforms and their hydraulic stability relationships in a tidal environment, Bay of Fundy, Canada

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Three intermediate- to large-scale bed configurations are recognised (from intertidal sand bodies in the Bay of Fundy), each with a discrete hydraulic stability field. Type 1 megaripples ('bars') form at lower flow velocities than Type 2 megaripples ('dunes'), whereas Type 2 megaripples and megarippled sandwaves are separated primarily by grain size. Megarippled sandwaves occur only in sands coarser than 0.308 mm.

TRANSVERSE sedimentary bedforms are produced by currents in many aqueous environments, and are of great interest to engineers and geologists. Because the complex nature of sediment-fluid interactions has prevented the development of a complete, theoretical understanding of bedform mechanics, attention has focused on empirical attempts to determine the hydraulic conditions governing bedform occurrence. Scaled-down flume experiments with steady-flow conditions have been used extensively¹⁻⁶. The results of such studies have greatly increased our knowledge of bedforms, but the much larger scale of most natural flows, and the unsteady conditions characterising them, limit the applicability of flume data to the real world. Complimentary bedform and hydraulic data from natural environments are needed.

This article presents a large body of such data, collected from intertidal sand bodies in the Bay of Fundy, Canada (Fig. 1). Bedform characteristics were recorded at approximately 760 locations and current velocity variations were monitored over complete tidal cycles at 110 stations. Nearly 3,000 vertical velocity profiles were measured. These data indicate that bedform development in this natural environment is more complex than that observed in flumes.

The study area

The investigations reported here⁷⁻⁹ were conducted in the eastern arm of the Bay of Fundy, on large intertidal sand bodies that occur in Cobequid Bay, the Avon River estuary, and along the north shore of the Minas Basin. The tides here are semidiurnal, anomalistic, and large, with a maximum measured range of 16.3 m at Burntcoat Head^{10,11}, and a mean range¹² of 11.9 m. Maximum water depths at high tide vary from 5 to 20 m, but depths at the times of peak current speed are generally between 2 and 10 m. The rate of water level rise and fall commonly exceeds 2 m h⁻¹.

Mean tidal current speeds ($\bar{U}$) vary greatly during each tidal cycle, and between neap and spring tides. The maximum speeds reach values of 0.5–2.0 m s⁻¹ during the middle to late part of the ebb tide, and in the first half of the flood. Cor-

responding maximum shear velocities fall between 0.03 and 0.15 m s⁻¹. The duration of peak flow strengths ranges from 1 to 4 h. The flow directions of the ebb and flood currents are generally 180° apart, but inequalities in the magnitude and/or duration of the opposing currents produce a persistent residual transport of sediment at most localities. Thus, either the ebb or flood portion of the tidal cycle dominates bedform formation and migration at any site.

The sediments on the sand bodies have mean sizes of 1.6–0.125 mm (–0.7 to 3.0 phi: very coarse to fine sand), but most are medium sand (0.5–0.25 mm: 1.0–2.0 phi). These sands exhibit good sorting, with standard deviations that are generally less than 0.8 phi units.

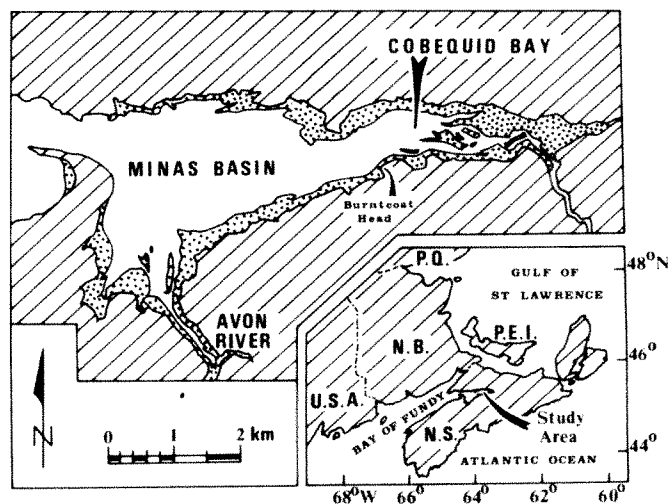
Additional information concerning the studied areas can be found in refs 7–9 and 13–16.

Bedform morphologies

Lower flow regime bedforms^{1,2} are abundantly developed on the sand bodies, and three basic types are recognised on the basis of size and morphology—ripples, megaripples, and sandwaves (Table 1). Upper flow regime plane bed^{1,2} is observed to occur during peak flow at only two locations in the Avon River estuary⁹.

Because of the great changes in flow direction, strength, and depth that occur during a tidal cycle, various modifications of the basic bedform types are produced^{7-9,13}, including: planing-off of megaripple crests in shallow flows; incomplete reversal of

Fig. 1 Map of the study area showing its location in the eastern arm of the Bay of Fundy (insert), and the extent of the intertidal zone at spring low tide (stippled pattern).



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Table 1 Summary of bedform size and morphological characteristics

Bedform type	Wavelength (<i>L</i>) (m)	Height (<i>H</i>) (m)	Steepness (<i>L/H</i>)	Morphological characteristics
Small scale: Ripples	<0.3	<0.05	~10	Straight to linguoid in plan Usually superimposed on larger forms as a late-stage modification
Intermediate scale: Type 1 Megaripples	0.1–25.0 (6.1)	0.05–0.50 (0.18)	10–150 (44.6)	Straight to smoothly sinuous in plan, without small sinuous irregularities Lack scour pits Height remains constant along crestline Flattened in section (<i>L/H</i> usually >20) Wavelengths and heights poorly correlated ($r = 0.462$ for $n = 70$), with a best-fit regression line of $H = 0.0947 (L)^{0.346}$ Less faces at angle of repose, producing tabular cross bedding
Type 2 Megaripples	0.05–14.0 (4.3)	0.05–0.70 (0.28)	6–34 (16.5)	Generally sinuous to lunate in plan, but may be straight with small sinuous irregularities Scour pits usually well developed Height variable along crestline Profiles are steep (<i>L/H</i> usually <20) Wavelengths and heights well correlated ($r = 0.788$ for $n = 255$), with a best-fit regression line of $H = 0.0865 (L)^{0.787}$ Less faces at angle of repose, producing trough cross bedding
Large scale: Megarippled sandwaves	10.0–215.0 (40.6)	0.15–3.4 (1.86)	17–210 (44.1)	Straight to smoothly sinuous in plan Scour pits absent Height constant along crestline Lee face inclination generally 10°–20° Wavelengths and heights moderately correlated ($r = 0.791$ for $n = 58$), with a best-fit regression line of $H = 0.0635 (L)^{0.733}$ Megaripples (usually Type 2, but less commonly Type 1) are superimposed on the sandwaves
Rippled sandwaves	5.0–25.0 (12.9)	0.15–0.75 (0.38)	30–55 (36.6)	Morphologically similar to megarippled sandwaves but only have ripples or lower flow regime plane bed superimposed on their stoss sides

Average values of height, wavelength and steepness index (*L/H*) are given in parentheses. r = correlation coefficient; n = no. of data points.

megaripples; and superposition of current ripples on all surfaces (Fig. 2) during the decelerating stages of the ebb tide. To simplify the analysis, and make the results more directly comparable with other environments, these modifications are not considered here.

Ripples are the smallest bedform type (Table 1) with heights and wavelengths of less than 0.05 m and 0.3 m respectively¹⁷. All shapes from straight to linguoid are observed. Ripples most commonly occur superimposed on the larger bedforms, but they are found on their own in a few localities. Only the latter are considered in the hydraulic section below.

Megaripples¹⁸ (Fig. 2a, b) are analogous to the dunes of most authors^{1,17,19}, and are intermediate in scale between ripples and sandwaves (Table 1). All megaripples have a lee face that is at the angle of repose of the sediment during active migration, but otherwise show a wide variation in detailed morphology. We have, however, arbitrarily subdivided them into two varieties—Type 1 and Type 2 megaripples. No single characteristic can be used to distinguish between the two types, and a continuous gradation occurs between them.

Type 1 megaripples are simple in appearance, and are characterised by straight to smoothly sinuous lee faces that lack irregularities with a wavelength of ~2–3 m (Fig. 2a). Scour pits are not present in the troughs; consequently, lateral continuity is good, and the height is very regular along an individual bedform. The wavelengths of these megaripples are long relative to the height, giving them flat profiles with wavelength/height ratios generally greater than 20 (Fig. 3). In addition, the heights and wavelengths of Type 1 megaripples are poorly correlated (Fig. 3, Table 1).

Type 2 megaripples are more complex in appearance (Fig. 2b), and are generally sinuous to lunate in plan. Some straight-crested examples do occur, but these exhibit small, sinuous irregularities along the lee face with a wavelength ~2–3 m. The troughs contain well-developed scour pits (Fig. 2b), commonly separated by prominent spurs. As a result, bedform height varies considerably over short distances along the crestline. Type 2 megaripples have steep profiles, and on average they are higher and shorter than Type 1 megaripples (Fig. 3, Table 1). The heights and wavelengths of Type 2 megaripples are quite well correlated (Table 1).

Sandwaves^{13,14,20,21} are the largest scale of bedform (Fig. 2c), with average heights and wavelengths markedly larger than those of megaripples (Fig. 3, Table 1). Most sandwaves are asymmetrical, but their lee faces commonly have inclinations of only 10–20°. (This is believed to be a result of the reversing flows, as possible fluvial counterparts possess avalanche faces^{22,23}.) Sandwaves display many similarities to Type 1 megaripples: they are straight to smoothly sinuous in plan; their troughs lack scour pits; the height remains uniform along a particular bedform (Fig. 2c); and they have flattened profiles with wavelength/height ratios rarely less than 20 (Fig. 3); but the heights and wavelengths of sandwaves are moderately well correlated (Table 1). Complete morphological gradations are observed between Type 1 megaripples and sandwaves, but no such gradational relationship is seen between sandwaves and Type 2 megaripples.

Virtually all sandwaves bear megaripples (Type 2 forms generally) on their stoss sides, producing 'megarippled sandwaves' (Fig. 2c). These megaripples do not appear to be a

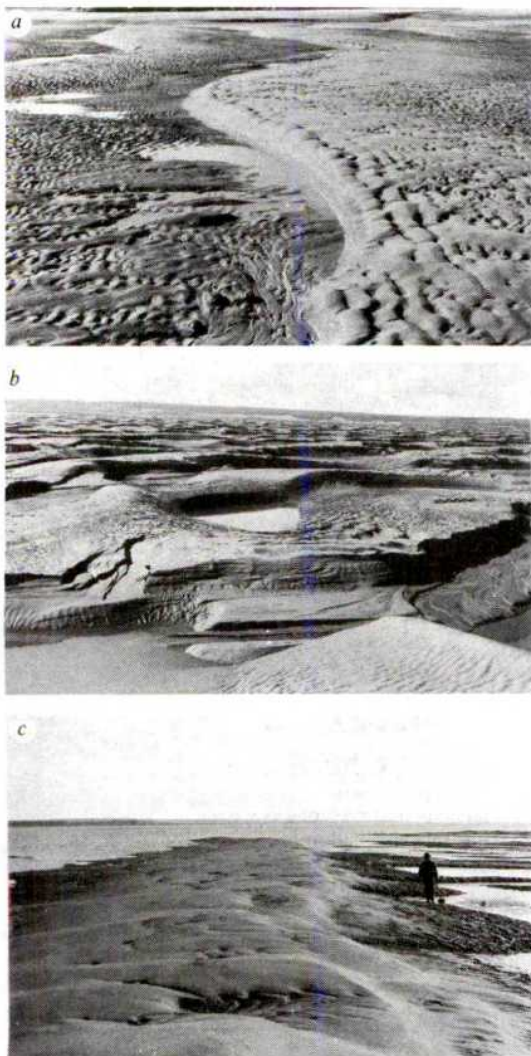


Fig. 2 Intermediate- and large-scale bedform types. *a*, Type 1 megaripples: note the simple plan geometry, absence of scour pits, and uniformity of bedform height (~ 0.2 m). *b*, Type 2 megaripples with well-developed scour pits and three-dimensional geometries. Metre stick at right for scale. *c*, Megarippled sandwaves: note the straight crestline and the lack of scour pits, other than those associated with the superimposed Type 2 megaripples. The megaripples on the sandwave crest have been partially rounded-off by wave action during emergence.

late-stage modification as are the superimposed current ripples. Instead, echo-sounder records show⁸ that the megaripples are present and active throughout both the semidiurnal and neap-spring tidal cycles at most localities. This megaripple-on-sandwave superposition seems, therefore, to represent an equilibrium co-existence, comparable to examples reported from both fluvial²² and shallow marine²⁴ settings.

In a few instances, only ripples or lower flow regime plane beds are present on the sandwave stoss sides. These forms, called rippled sandwaves, are smaller on average than megarippled sandwaves (Table 1), but are otherwise similar in morphology. Observations suggest that rippled sandwaves may be a disequilibrium form of megarippled sandwaves, because the former almost always occur in situations of decelerating flow: either as a neap-tide replacement of megarippled sandwaves that exist during spring tides; or as a transitional form between megarippled sandwaves and Type 1 megaripples, produced by bedform migration into an area of lower current strength and shallower water depth. In both cases, the superimposed megaripples disappear before the sandwave because of their smaller size and shorter lag time^{25,26}. During the prograde transition from Type 1 megaripples to mega-

rippled sandwaves, a gradual increase in bedform size commences at the same time as the superimposed megaripples appear, and rippled sandwaves do not occur as an obvious intermediate step. These observations may support experimental work suggesting that bedform destruction is a less 'efficient' and slower process than bedform generation²⁷.

Hydraulic relationships

Bedforms respond in some manner to all flows that are above the threshold of sediment movement, but lag behind changes in flow conditions because of the finite time required to reach a new equilibrium^{25,26}. As a result, it is difficult to determine a specific set of hydraulic conditions responsible for the bedforms observed in the study area, because flow conditions vary greatly over the semidiurnal tidal cycle. It is thought, however, that a reasonable approximation to these 'effective' conditions is obtained by assuming that the major bedforms are produced by the peak flow during the dominant half of the tidal cycle. This is the period when the largest amount of sediment is transported, and data⁸ on the variation of friction factors over the tidal cycle suggest that the bedforms approach equilibrium most closely at this time.

Therefore, representative 'average-maximum' values of the mean current speed ($\bar{U}$) have been calculated by averaging the three highest mean speeds measured in water depths greater than 2.5 m during the dominant half of each monitored tidal cycle. (The depth limit of 2.5 m was established because fast currents in shallower flows were observed to plane-off pre-existing bedform crests.) Average-maximum values of shear velocity (U_*) and stream power (ω) also were calculated in the same manner. The mean water depth was taken as the average of the depths in which the three maximum $\bar{U}$ values occurred. All of these data are summarised in Table 2, and phase diagrams involving water depth, mean current speed and grain size are shown in Fig. 4.

Figure 4 shows that a good separation of the bedform types is achieved using the above procedure. Some overlap does occur, but this is to be expected because of the complexity of the environment as compared to closely controlled flume experiments. One factor not previously considered that contributes to the overlap in terms of $\bar{U}$ is the variation of flow strength with tidal range. During neap and spring tides, the maximum current speeds may, for a few tidal cycles, fall outside the stability range of the bed configuration developed by the preceding average tides. Because of lag effects^{25,26}, the bedforms do not always achieve a new equilibrium, and thus exist 'metastably'

Fig. 3 Plot of bedform height (H) against wavelength (L). Envelopes to the observed values are given for Type 1 megaripples, Type 2 megaripples and megarippled sandwaves. The thin diagonal lines are contours of the steepness index (L/H). $\circ$, Type 1 megaripples; $\bullet$, Type 2 megaripples; $\square$, rippled sandwaves; $\blacksquare$, megarippled sandwaves.

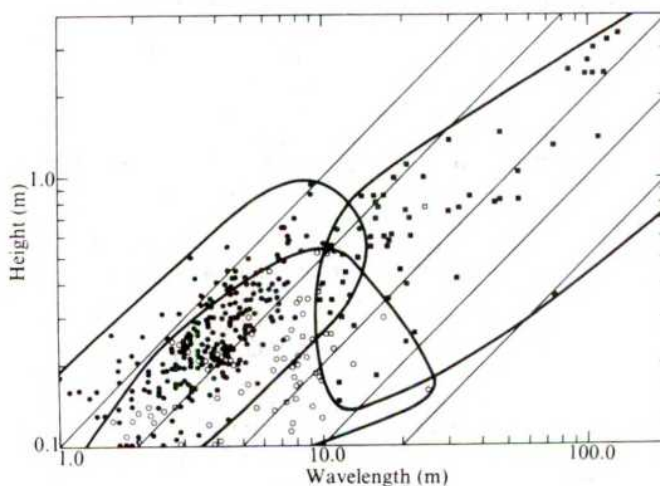
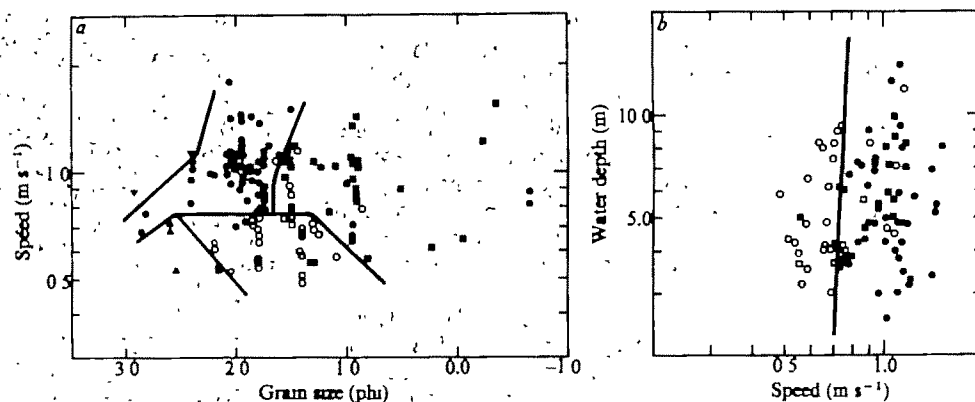


Fig. 4 Bedform phase diagrams, constructed by the method outlined in the text. The dominant half of the tidal cycle was established by comparing average-maximum values of $\bar{U}$ for the ebb and flood. The bed configuration developed at each site was determined by direct observation during emergence and/or from echo-sounder records. Δ , Ripples; $\circ$, Type 1 megaripples; $\bullet$, Type 2 megaripples; $\square$, rippled sandwaves; $\blacksquare$, megarippled sandwaves; ∇ , upper-flow regime plane bed. **a**, Average-maximum mean current speed plotted against mean grain size. Data for all water depths are included. **b**, Mean water depth plotted against average-maximum mean speed. Only data for medium sand (0.5–0.25 mm; 1.0–2.0 phi) are included.



out of their normal stability field, thereby creating overlap. However, this does not obscure the basic hydraulic relationships.

In the study area, ripples occur alone, without larger bedforms, only where the mean sediment size is <0.25 mm (2.0 phi) and the average-maximum current speed is below 0.75 m s^{-1} (Fig. 4a). The upper flow regime plane bed field also is restricted to fine grain size (less than ~ 0.21 mm: 2.25 phi) within the range of velocities encountered.

Type 1 megaripples form in sands coarser than 0.18 mm (2.5 phi) at average-maximum mean speeds comparable to those producing ripples, and at flow strengths (as expressed by either $\bar{U}$, u_{*} , or ω) which are considerably below those producing Type 2 megaripples (Fig. 4a; Table 2). This flow-strength distinction between straight-crested (Type 1) and three-dimensional (Type 2) megaripples has been known qualitatively for some time¹⁷, but has not previously been quantified. The degree of separation of the two megaripple types in Fig. 4 is remarkable, given the gradational nature of the morphological transition between them. This indicates that the distinction used here is not only consistent, but also of potential genetic significance.

Megarippled sandwaves also form at higher flow strengths than Type 1 megaripples, but at a slightly lower average strength than Type 2 megaripples (Fig. 4a; Table 2). However, the overlap between megarippled sandwaves and Type 2 megaripples in terms of $\bar{U}$ (and also u_{*} and ω) is large (Fig. 4); and both bedform types form in an almost identical range of water depths (Fig. 4b), a finding which sharply disagrees with the results of a previous study²⁸. Clearly, mean grain size is the only major variable that distinguishes between these two bed configurations (Fig. 4a): sandwaves are restricted to sediments coarser than approximately 0.308 mm (1.70 phi); whereas

megaripples coexist with sandwaves at those grain sizes but occur alone in finer sands. (Some Type 2 megaripple points plot within the megarippled sandwave stability field in Fig. 4a, but most of these represent starved megaripples migrating over gravel lag or bedrock. It may be that sandwaves, like desert draas²⁹, require a minimum thickness of mobile sediment before their formation is possible.)

Rippled sandwaves have not been given a separate stability field in Fig. 4a and b, because they overlap considerably with Type 1 megaripples and to a lesser extent with megarippled sandwaves. This behaviour agrees with the earlier suggestion that rippled sandwaves are a retrograde form of megarippled sandwaves.

Discussion

Comparison of the current speed-grain size phase diagram developed for this natural environment (Fig. 4a) with those constructed from shallow (0.2–0.4 m) flume experiments^{6,30,31} shows a close similarity in the positioning and slope of the various phase boundaries, despite the vastly different flow depths. The flume studies reveal that the stability field of dunes gradually pinches out as the sediment size decreases, so that dunes do not occur at grain sizes finer than approximately 0.1 mm (3.3 phi)³⁰. If Type 1 and Type 2 megaripples are considered together, then a similar pattern is evident in Fig. 4a. In addition, the slope of the Type 1 megaripple phase boundaries in Fig. 4a matches that of 'sand waves' in ref. 31 over the corresponding range of grain sizes.

These similarities, plus the near-vertical orientation of the Type 1–Type 2 megaripple boundary in Fig. 4b, are clear evidence that water depth has little influence on bedform development, especially in flow depths as great as those encountered in the study area.

Table 2 Mean values of sediment and flow parameters characterising the occurrence of the various bedform types

Bedform type	Mean grain size (mm)	Average-maximum mean speed (m s^{-1})	Mean water depth (m)	Average-maximum shear velocity (m s^{-1})	Average-maximum stream power ($\text{kg s}^{-1} \text{m}^{-1}$)
Ripples	0.184 (2.44 ϕ)	0.603	5.37	0.035	0.739
Type 1 Megaripples	0.328 (1.61 ϕ)	0.687	5.57	0.044	1.800
Type 2 Megaripples	0.272 (1.88 ϕ)	1.052	6.00	0.082	7.628
Megarippled sandwaves	0.507 (0.98 ϕ)	0.955	6.45	0.065	4.748
Rippled sandwaves	0.369 (1.44 ϕ)	0.701	4.50	0.045	1.750

Examination of the hydraulic and morphological data indicates that Type 1 megaripples are equivalent to the 'bars' described by Costello⁶, whereas Type 2 megaripples represent the dunes of refs 6, 30, and 31. Confusion arises, however, because 'bars' are, unfortunately, also called 'sand waves', and are thought^{6,30,31} to be a smaller-scale equivalent of the large sandwaves reported from many natural environments^{13,20-22}. The positioning of the distinct megarippled sandwave field in Fig. 4a does not seem to support such a comparison, despite the many morphological similarities between sandwaves and Type 1 megaripples ('bars').

It has been suggested^{32,33} that sandwaves are large dunes, which are in equilibrium with deeper and faster flows than smaller dunes. The large overlap of the megarippled sandwave and Type 2 megaripple stability fields in terms of both speed and depth (Fig. 4a, b) strongly indicates that this interpretation is not valid, at least in the study area. The formation of megaripples and sandwaves in similar water depths (Fig. 4b, Table 2) also calls into question the generality of the commonly cited relationship between water depth and bedform wavelength³⁴, which forms the basis of the computational model of bedform lag developed recently by Allen^{33,35}. The results of such models must, therefore, be treated with caution until more is known about sandwaves and their genetic relationship to 'bars' and dunes. Clearly, further detailed study in natural environments is necessary until such time as sandwaves can be reproduced in flumes.

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Transformation of yeast by a replicating hybrid plasmid

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Chimaeric plasmids have been constructed containing a yeast plasmid and fragments of yeast nuclear DNA linked to pMB9, a derivative of the ColEI plasmid from E. coli. Two plasmids were isolated which complement leuB mutations in E. coli. These plasmids have been used to develop a method for transforming a leu2 strain of S. cerevisiae to Leu⁺ with high frequency. The yeast transformants contained multiple plasmid copies which were recovered by transformation in E. coli. The yeast plasmid sequence recombined intramolecularly during propagation in yeast.

PROCEDURES for cloning DNA segments in *Escherichia coli* by insertion into a plasmid or bacteriophage genome are now well established and have been used to isolate many prokaryotic and eukaryotic DNA sequences. However, many eukaryotic genes do not have counterparts in bacteria and therefore cannot be isolated by complementation of a bacterial deficiency, and

sequences involved in regulation or development may only be usefully studied in a homologous genetic background.

A yeast cloning system would be extremely useful for the isolation of genes and regulatory sequences from yeast and other eukaryotic organisms and for the genetic manipulation of commercially important yeasts. Recently yeast cells were transformed at a frequency of 10^{-7} transformants per viable cell using a derivative of the bacterial plasmid ColEI carrying the yeast *LEU2* gene which codes for β -isopropylmalate dehydrogenase¹. The transforming plasmid was apparently not present in yeast clones in the free or non-integrated form but was integrated into yeast chromosomes at more than one site.

I describe here the development of a highly efficient yeast transformation system using yeast-bacterial hybrid plasmids which are able to replicate and may be selected in and recovered from both *E. coli* and *Saccharomyces cerevisiae*. The hybrid plasmids incorporate a yeast plasmid, 2 μ m long (approximately 6 kilobases)²⁻⁵ which was previously cloned in bacteriophage λ and analysed by restriction endonuclease digestion to determine its suitability as a cloning vehicle⁶.

The yeast plasmid is present in many strains of *S. cerevisiae*

Fig. 1 Plasmid pMB9, the 2- μ m plasmids from *S. cerevisiae*, and the hybrid plasmids used to clone nuclear DNA fragments from *S. cerevisiae*. The physical map of pMB9 is essentially that described by Bolivar *et al.*¹⁵ and the *Hpa*I endonuclease target was mapped in this work. The tetracycline resistance gene is represented by Tc^r. The *S. cerevisiae* plasmid types A and B which are presumably interconvertible are represented as dumbbells with the inverted repeat regions corresponding to the stem portion. The sizes of *Eco*RI restriction digest fragments are given as kilobases and were reported previously⁶. The structures are shown of the four hybrid plasmids chosen for further cloning experiments out of the eight possible configurations. To construct these hybrid plasmids, purified¹⁶ yeast plasmid DNA was restricted with *Eco*RI endonuclease¹⁹ under conditions such that digestion was incomplete and the majority of the products were in the form of full length linear molecules as determined by agarose gel electrophoresis¹⁹. Plasmid pMB9 DNA purified from chloramphenicol-treated cultures²⁰ by a cleared lysate procedure²¹ was digested with *Eco*RI endonuclease and the two digested DNA samples were mixed and ligated²². *E. coli* strain ED8767 (*recA56 metB⁻ hsdS⁻ supE supF*; W. J. Brammar)²² was made competent by calcium chloride starvation²³ and transformed by heat shock treatment with DNA at 0.3 μ g ml⁻¹. Cells were diluted threefold with L-broth and incubated at 37 °C for 40 min before plating to select for transformants on fresh L-broth agar containing tetracycline hydrochloride at 15 μ g ml⁻¹. Transformant clones were screened for the presence of the yeast plasmid sequence by *in situ* hybridisation²⁴ with a ³²P-labelled probe prepared by nick translation²⁵ of DNA from a lambda clone of the yeast plasmid⁶. Clones of plasmids containing the yeast sequence were further analysed to identify those in which the complete yeast plasmid was present. The size of plasmid in each clone was estimated by gel electrophoresis of colony lysates²⁶. Clones with full-size hybrid plasmids were further characterised by restriction analysis of the plasmid DNA.

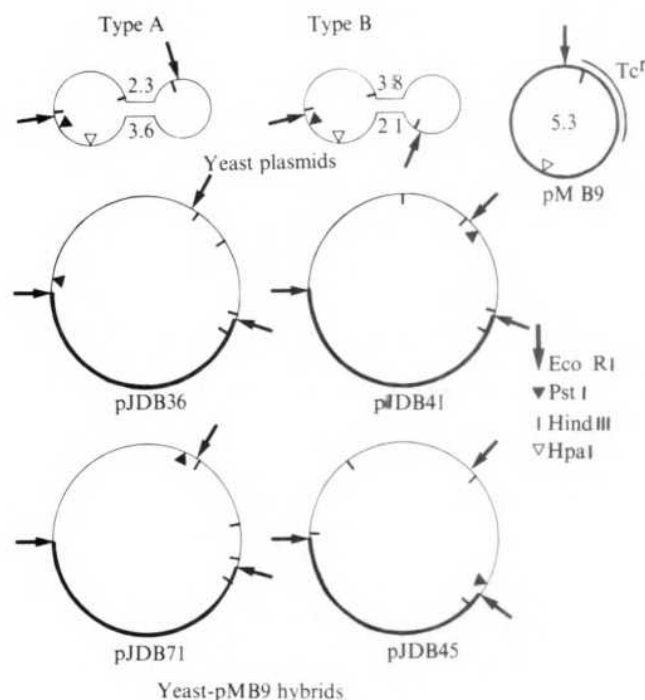
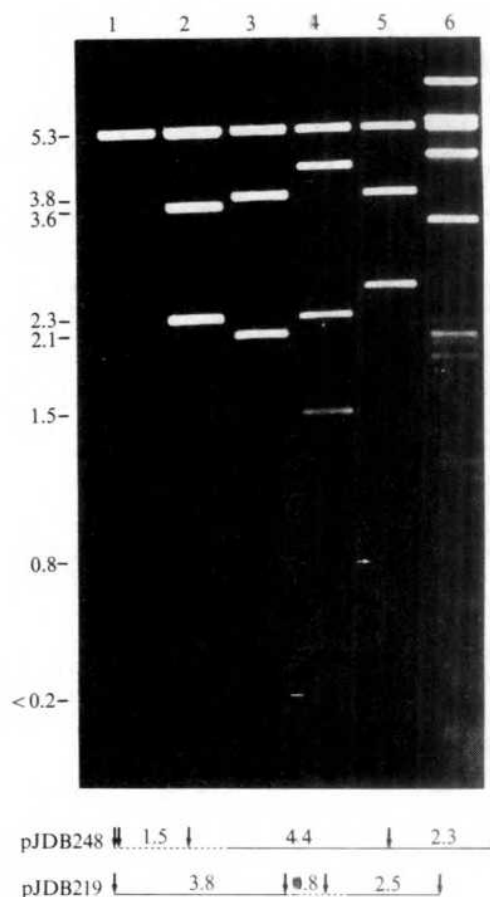


Fig. 2 Cloning of *leuB*-complementing sequences from yeast nuclear DNA into hybrid plasmids. *Eco*RI endonuclease digests of (1) pMB9 DNA, (2) pJDB36 (type A hybrid plasmid) DNA, (3) pJDB41 (type B hybrid plasmid) DNA, (4) pJDB248 (type A *leuB*⁺ plasmid), (5) pJDB219 (type B *leuB*⁺ plasmid) and (6) *Eco*RI endonuclease digest of λ DNA mixed with a *Hpa*I endonuclease digest of λ DNA. DNA fragment sizes are given in kilobases and faint bands are indicated by arrows. In the diagram only yeast sequences in pJDB248 and pJDB219 are shown. The yeast plasmid sequence is represented by a continuous line and the yeast nuclear DNA inserts by discontinuous lines. Arrows in the diagram represent sites for *Eco*RI endonuclease. Nuclear DNA (a gift from C. Christiansen) from *S. cerevisiae* strain M127 (prototrophic) was sheared in a Virtis 60K homogeniser²⁷ such that the major fraction of sheared DNA was in fragments 6 to 15 kilobases long with a smaller proportion of fragments of length ranging from less than 1 kilobase. A mixture of the DNAs of the hybrid plasmids pJDB36, pJDB41, pJDB45 and pJDB71 was digested with *Pst*I endonuclease. Both the yeast and the plasmid DNAs were extracted with phenol, extracted with ether and extensively dialysed against 0.1 M potassium cacodylate pH 7.0. Poly(dA) tails were added to the yeast DNA and poly(dT) tails were added to the plasmid DNAs by incubation with calf thymus terminal deoxynucleotidyl transferase. The reaction conditions were as described by Lobban and Kaiser²⁸, except that the temperature of the poly(dT) reaction was lowered to 0 °C, and in both reactions MgCl₂ was replaced by 0.5 mM CoCl₂ (ref. 29). The reactions were stopped by the addition of EDTA when 50 nucleotides had been incorporated per 3' terminus. The tailed DNAs (0.37 μ g of each DNA ml⁻¹) were annealed in 10 mM Tris, 1 mM EDTA, 100 mM NaCl pH 8 by incubating at 65 °C for 10 min and allowing to cool to room temperature in the same water bath. The DNA was cloned in *E. coli* JA221 by selection of tetracycline-resistant transformants. A total of 21,000 clones were stored in batches of 500 at -80 °C in 12.3 g per l K₂HPO₄, 0.9 g per l sodium citrate, 0.18 g per l MgSO₄·7H₂O, 1.8 g per l (NH₄)₂SO₄, 3.6 g per l KH₂PO₄, 88 g per l glycerol (D. Hogness, personal communication). In addition 5,000 of these clones were maintained individually in L-broth containing 7% dimethylsulphoxide in microtitre plates at -20 °C. Clones with plasmids complementing the *leuB6* mutation in *E. coli* JA221 were selected by growth of samples from the batches of clones on minimal selective plates (the minimal medium of Spizizen³⁰ was used with 0.2% w/v glucose, 1.5% Difco Bacto agar and 20 μ g ml⁻¹ L-tryptophan). The *leuB6*-complementing plasmids (pJDB219 and pJDB248) were analysed by digestion of the DNAs with *Eco*RI, *Hind*III, and *Hpa*I endonucleases and gel electrophoresis to determine the configuration of the hybrid plasmid in each case. Electrophoresis was through a 1% agarose horizontal slab gel (18 × 24 × 0.5 cm) in 40 mM Tris, 20 mM sodium acetate, 1 mM EDTA pH 8.2 at 100 V per 30 mA for 15 h. The gel was stained by soaking in a solution of ethidium bromide (0.5 μ g ml⁻¹) and photographed with a UV light source below.



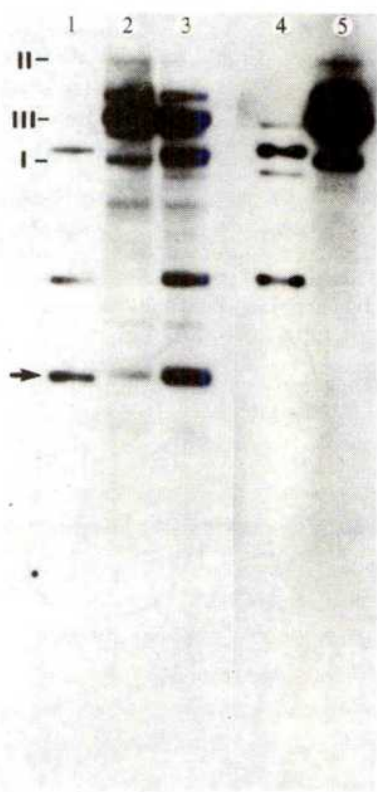


Fig. 3 Autoradiograph of ^{32}P -labelled cRNA prepared³¹ to *EcoRI* digested DNA of pJDB248 (1, 2 and 3) or pJDB36 (4 and 5) hybridised with *EcoRI* digested pJDB248 DNA (1 and 4), *S. cerevisiae* DNA (2 and 5) or *S. cerevisiae* and pJDB248 DNAs together (3). The bands corresponding to the 1.5 kilobase DNA fragment are indicated by an arrow. The slower migrating bands (I, II, III) observed in the yeast DNA digests correspond to the supercoiled, nicked-circular and linear forms respectively of 2- μm yeast plasmid which was relatively resistant to digestion by *EcoRI* endonuclease although there were two targets for this enzyme in the plasmid sequence of this strain. Digestion of plasmid DNA with *EcoRI* endonuclease before the preparation of cRNA had the effect of increasing the efficiency of the RNA polymerase reaction, but the yeast sequences were then preferentially transcribed, resulting in the relatively lower intensity of the band due to pMB9 on the autoradiograph. The *S. cerevisiae* DNA used in this experiment was a different preparation from that used in the cloning experiment (and was prepared as described in the legend to Fig. 5). The possibility that the cloned DNA fragment was a contaminant in the DNA preparation is therefore unlikely. *EcoRI* digested DNAs were electrophoresed through a 1.5% agarose slab gel at 120 V per 45 mA for 13 h and transferred³² to a nitrocellulose filter (Millipore HAWP). Hybridisation of the filters was carried out at 60 °C in 4 ml $2\times\text{SSC}$ (0.03 M sodium citrate, 0.3 M sodium chloride, pH 7.0) containing 20 μg *E. coli* tRNA and cRNA (300,000 d.p.m.) with rapid shaking for 16 h. The filters were washed in $1\times\text{SSC}$ at 60 °C with gentle shaking for 2 h. The sensitivity of Kodak X-Omat H film was enhanced by using Ilford fast tungstate screens and storing at -80 °C during the exposure³⁵.

but has no known function. The plasmid has the same density as main band nuclear DNA^{2,5} and replicates under nuclear control^{7,8}, there being 50 to 100 plasmid copies per cell⁹. It contains an inverted repeat sequence 600 base pairs long and is isolated as a mixture of two forms which differ from one another in the orientation of the two nonrepeated segments of the molecule with respect to each other. The two forms are presumably due to recombination between the inverted repeat sequences¹⁰⁻¹². Plasmids isolated from several different strains of *S. cerevisiae* differ from each other by small deletions or point mutations which alter the restriction enzyme cleavage pattern of the DNAs^{13,14}.

Construction of chimaeric plasmids

The plasmids were constructed in two stages. First, pMB9, a derivative of the ColEI plasmid from *E. coli*¹⁵, and the plasmid from *S. cerevisiae* DR19/8T¹⁶ were joined by ligation of the DNA fragments produced by *EcoRI* endonuclease (Fig. 1). The pMB9 sequence includes a tetracycline resistance determinant and tetracycline-resistant clones were selected after transforming *E. coli* with the ligated DNAs. These clones were screened for a hybrid plasmid large enough to carry the complete yeast plasmid sequence.

Hybrid yeast-pMB9 plasmids theoretically have eight possible configurations. These are determined by whether the yeast sequence is in type A or type B configuration, by which of the two *EcoRI* endonuclease targets joins the sequence to pMB9, and by the orientation of insertion into pMB9. These configurations can be differentiated by the digestion pattern of the plasmid DNA with *HindIII* or *HpaI* endonucleases. Five of these different configurations were found amongst seven complete hybrid plasmids examined. The significance of the two forms (A and B) of yeast plasmid was not known. Nor was it known whether cloning DNA fragments into either of the *EcoRI* sites of the yeast plasmid would inactivate a sequence required for the maintenance of the plasmid in yeast or for some other essential function. Therefore, four of the different hybrid plasmids, pJDB36, pJDB41, pJDB45 and pJDB71 (Fig. 1), were used in the second stage of the construction of the plasmids to be used in the transformation of yeast.

Sheared nuclear DNA from *S. cerevisiae* of average fragment length 6 to 15 kilobases was linked by means of poly(dA-dT) tails¹⁷ to a mixture of DNAs of pJDB36, pJDB41, pJDB45 and pJDB71 which had been converted to linear molecules by digestion with *PstI* endonuclease. Tetracycline resistant clones were selected after transformation of *E. coli* JA221 (*recA1*, *leuB6*, *trpΔE5*, *hsdR⁻ hsdM⁺ lacY C600*; J. Carbon) with the annealed DNAs. A total of 21,000 clones were obtained, and those which were phenotypically *leu⁺* were isolated by selection on minimal plates. Two plasmids, pJDB219 and pJDB248, were isolated which contained a sequence able to complement the *leuB6* mutation in strains JA221 and C600. Both plasmids transformed strain JA221 to *leu⁺* or to tetracycline resistance with equal efficiency (10^5 transformants per μg DNA). The plasmids also complemented the *leuB61* and *leuB401* alleles¹⁸ at the same frequency as transformation to tetracycline resistance in the same strains.

Plasmid pJDB248 was pJDB36 with an insertion of approximately 2.4 kilobases containing two sites for *EcoRI* endonuclease which were 1.5 kilobases apart (Fig. 2). Plasmid pJDB219 was formed by an insertion of approximately 1.2 kilobases (containing one target for *EcoRI* endonuclease) in pJDB41.

Minor bands corresponding to DNA sequences of 3.8 and 2.9 kilobases were observed in varying amounts in *EcoRI* digests of different preparations of pJDB248 DNA from cultures produced from purified colonies. These corresponded to the fragment sizes expected if pJDB248 contained the type B yeast plasmid sequence rather than type A. Presumably the yeast inverted repeat sequences recombined during propagation in *E. coli*. This recombination was not observed in other *E. coli*-yeast hybrid plasmids. However, the rate of recombination may be slow relative to the growth rate of *E. coli*, and the growth of cultures from original clones was not extended beyond the minimum required for preparation of plasmid DNA.

Figure 3 shows that DNA from *S. cerevisiae* digested with *EcoRI* endonuclease contained a 1.5 kilobase sequence which hybridised to the ^{32}P -labelled cRNA prepared to pJDB248 DNA, but not to cRNA prepared to pJDB36. This indicates that the inserted sequence corresponds to a yeast DNA fragment. The same *EcoRI*-generated DNA fragment hybridised cRNA prepared to pJDB219, indicating that the *leuB*-complementing sequences in the two independently isolated plasmids were the same.

Transformation of yeast

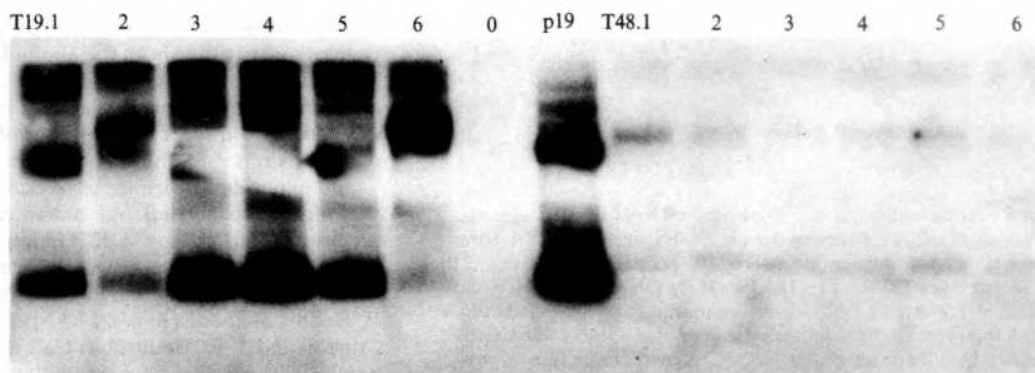
S. cerevisiae MC16 α leu2-3 his4-712^{FS} ade2-1 lys2-1 SUF2; G. R. Fink (FS denotes a suppressible mutation, SUF2 is a suppressor gene) was used as the recipient strain to investigate the uptake and replication of the plasmids and the transfer of the Leu⁺ phenotype. Protoplasts and DNA were mixed and treated with polyethylene glycol to promote DNA uptake (see legend to Fig. 4). In different experiments between 10% and 30% of the cells thus treated regenerated the cell wall and produced colonies in supplemented minimal agar. Viable protoplasts were transformed to LEU⁺ by pJDB219 or pJDB248 DNA with a frequency of 5×10^{-4} to 3×10^{-3} transformants per viable cell. The leu2 mutation in this strain reverted to LEU⁺ at a frequency of only 10^{-8} to 10^{-7} . Clones transformed by pJDB248 grew as fast on selective plates as the untransformed cells grew on supplemented plates: protoplasts gave rise to colonies in 2 to 3 days. Transformants of pJDB219 required 3 to 4 days to develop colonies. [Note, efficiency of transformation of *S. cerevisiae* was not linearly proportional to DNA concentration, and a saturating DNA concentration was not found. Transformation frequencies of 10^4 to 10^5 transformants per μ g DNA were obtained in the conditions used here.]

To detect the presence of the transforming plasmids in yeast clones, extracts of yeast cells were analysed by gel electrophoresis, transfer of the DNA to nitrocellulose and hybridisation with a ³²P-labelled cRNA prepared to pMB9 DNA. Transformants T19.1 to T19.6 (Fig. 4) produced bands hybridising to the pMB9 sequence and with similar electrophoretic mobility to the transforming plasmid pJDB219 (upper and lower bands correspond to nicked circular and supercoiled molecules respectively, slower migrating minor bands probably represent

oligomeric forms of the plasmid). Transformants T48.1 to T48.6 also contained plasmids with the pMB9 sequence and with lower electrophoretic mobility than pJDB219, as expected for the larger plasmid pJDB248 (not shown in Fig. 4). There were apparently more copies per cell of pJDB219 than of pJDB248 in their respective transformant clones.

To investigate the integrity of the plasmid sequence in transformants, DNA was extracted and analysed by digestion with *Eco*RI endonuclease. Figure 5 shows the banding pattern of *Eco*RI-digested plasmid DNA isolated from two yeast clones transformed by pJDB219. The bands found in digests of pJDB219 DNA (corresponding to 5.3, 3.8, 2.5 and 0.8 kilobase DNA fragments) can be seen in the transformant DNA digests, and also the bands found in the digests of 2- μ m plasmid DNA (3.8, 3.6, 2.3 and 2.1 kilobase fragments). There is also a band in the transformant DNAs corresponding to a 4.0 kilobase fragment which would be expected if the yeast plasmid in pJDB219 recombined during propagation in yeast, converting a type B to a type A plasmid (fragments of length 3.8 and 2.5 kilobases would be replaced by fragments of length 4.0 and 2.3 kilobases). Thus all the bands in the plasmid DNA of one transformant (Fig. 5, track 2) are those expected for a mixture of the 2 μ m plasmid and pJDB219, however, two more bands can be seen in the plasmid DNA of the other transformant (Fig. 5, track 3) corresponding to 3.4 and 1.9 kilobase fragments. These bands cannot be readily accounted for. They may be products of recombination between plasmids¹¹, or of specific deletion events. Two examples of each type of banding pattern were found in digests of DNA from four different yeast clones transformed by pJDB219. The DNA from three yeast clones transformed by pJDB248 DNA was similarly analysed. The

Fig. 4 Autoradiograph of ³²P-labelled cRNA to pMB9 DNA hybridised with undigested DNA of lysates of yeast clones transformed with pJDB219 (clones T19.1 to T19.6) or pJDB248 (clones T48.1 to T48.6) and in the lysate of the untransformed recipient strain alone (0) and with pJDB219 DNA (p19). Cultures of *S. cerevisiae* MC16 to be transformed were grown on YPDA



(1% yeast extract, 2% Difco bacto-peptone, 2% glucose, 20 mg l⁻¹ adenine) and collected at 10^8 cells ml⁻¹. Cells were washed in sterile distilled water, concentrated threefold in 1.2 M sorbitol, 25 mM EDTA, 50 mM dithiothreitol pH 8 and incubated at 29 °C for 10 min with gentle shaking. Cells were washed twice by centrifugation and resuspension in the original culture volume of cold, sterile 1.2 M sorbitol. Protoplasts were obtained by concentrating the cells threefold in 1.2 M sorbitol, 0.01 M EDTA, 0.1 M sodium citrate pH 5.8 containing Helicase lyophilised snail gut extract at 2 mg ml⁻¹ (Industrie Biologique Francaise; 2 g powder containing 10^6 units β -glucuronidase activity were dissolved in 10 ml distilled water, filter sterilised and stored at -20 °C), and incubated at 29 °C with gentle shaking for 1 h. Protoplasts were washed by centrifugation and resuspension in the original culture volume of 1.2 M sorbitol three times to remove nuclease activities present in the snail gut extract, and resuspended in 1.2 M sorbitol 10 mM CaCl₂ to give approximately 10^{10} protoplasts ml⁻¹. DNA was mixed with 0.1 ml or 0.2 ml protoplasts to give 10 to 20 μ g DNA ml⁻¹ (DNA samples were sterilised immediately before use by extraction with chloroform and extraction with ether). Protoplasts and DNA were left at room temperature for 15 min and then diluted with 10 volumes of 20% polyethylene glycol 4000, 10 mM CaCl₂, 10 mM Tris-HCl pH 7.5 (a modification of a protoplast fusion procedure³³). After 15 to 20 min protoplasts were pelleted and resuspended in 100 μ l 1.2 M sorbitol, 10 mM CaCl₂, 20 μ g ml⁻¹ leucine plus 50 μ l YPDA containing 1.2 M sorbitol, and incubated at 29 °C for 20 min. Samples were diluted in 1.2 M sorbitol and plated by mixing in 7 ml minimal medium containing 1.2 M sorbitol and 3% agar at 45 °C (minimal medium was 0.67% Difco yeast nitrogen base without amino acids, 2% glucose, 20 mg l⁻¹ adenine, lysine and histidine) and pouring onto minimal medium plates containing 1.2 M sorbitol, 2% agar. Dilutions were plated on supplemented (20 μ g leucine ml⁻¹) minimal plates to measure the efficiency of protoplast regeneration. Colonies developed after 2 to 4 days incubation at 29 °C. To test for the presence of the transforming plasmid, single clones were purified on minimal selective plates. Single colonies were used as inocula to make 1 cm $\times$ 2 cm streaks on YPDA plates which were incubated at 29 °C overnight. These streaks of cells were resuspended in 0.5 ml 1.2 M sorbitol, 25 mM EDTA, 0.86 M 2-mercaptoethanol, pH 8, left at room temperature for 10 to 20 min and centrifuged. The pelleted cells were resuspended in 0.5 ml 1.2 M sorbitol, 0.1 M sodium citrate, 10 mM EDTA pH 5.8 containing Helicase snail gut extract at 2 mg ml⁻¹ and incubated at 29 °C for 1.5 h. Protoplasts were washed twice by centrifugation and resuspension in 1.5 ml 1.2 M sorbitol and finally resuspended in 200 μ l 10 mM Tris, 1 mM EDTA, 1% sodium dodecyl sulphate pH 8. Protoplasts were lysed by incubation at 60 °C for 20 min and debris was pelleted. Lysate (40 μ l) was mixed with 20 μ l 20% Ficoll, 0.05% bromophenol blue and electrophoresed through 1% agarose slab gels at 120 V per 35 mA for 15 h. Gels were exposed to short wavelength ultraviolet light for 15 min to nick the supercoiled plasmids and transferred to nitrocellulose filters as usual³², except that the denaturation step was carried out at 37 °C for 1 h to ensure plasmid denaturation and to hydrolyse RNA. The filters were hybridised with ³²P-labelled cRNA (300,000 d.p.m.) as described in Fig. 2.

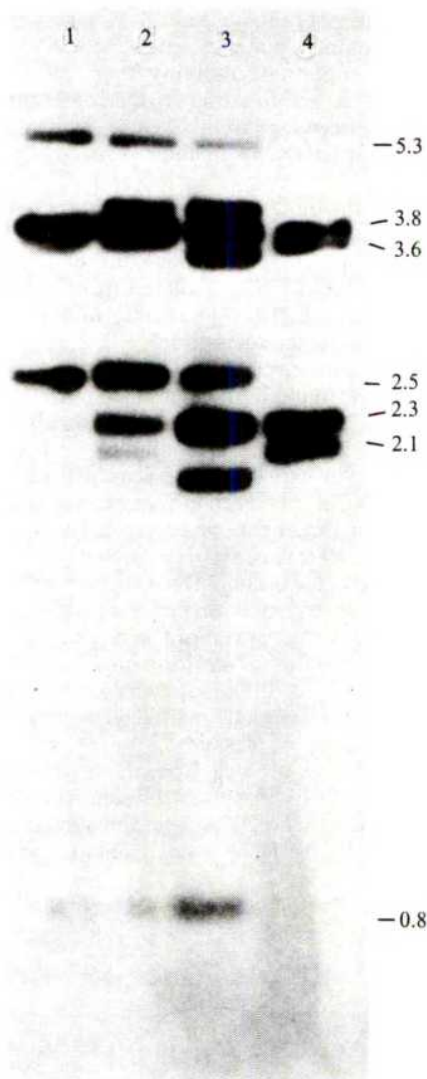


Fig. 5 Autoradiograph of ^{32}P -labelled cRNA to *Eco*RI digested DNA of pJDB219 hybridised with *Eco*RI digested DNA from yeast clones transformed by pJDB219. (1) pJDB219 DNA; (2) DNA from yeast clone T19.1 in Fig. 4; (3) DNA from yeast clone T19.2 in Fig. 4; (4) DNA from the untransformed yeast strain. DNA fragment sizes are indicated in kilobases. DNA was partially purified from cultures grown on YPDA medium by lysis of protoplasts³⁴ and extraction of the lysates with phenol and chloroform. Nucleic acids were ethanol precipitated, resuspended, and RNA was digested by pancreatic ribonuclease³⁴. The samples were finally dialysed against 5 mM Tris, 0.5 mM EDTA pH 8.

unexpected 3.4 and 1.9 kilobase fragments were produced from the DNA of two of these clones (not shown).

Recovery in *E. coli* of plasmids from yeast transformants

Samples of DNA (prepared as described in Fig. 5) from six pJDB219 transformants were used to transform *E. coli* JA221. Transformed cells were plated on minimal agar, minimal agar containing tetracycline ($15 \mu\text{g ml}^{-1}$) or leucine-supplemented agar containing tetracycline. No significant difference was found between the transformation frequencies for the *leuB* and the tetracycline resistance markers selected either independently or together. This suggests that the plasmids recovered from yeast transformants by complementation in *E. coli* had retained both the tetracycline resistance and the *leuB*-complementing sequences. This test gives no information about plasmids which may have retained the *leuB*-complementing sequence but lost

the pMB9 sequence, as the yeast plasmid probably does not replicate in *E. coli* (J. D. B., unpublished result). Similar results were obtained in recovering DNA from pJDB248 transformants although the level of complementation was reduced probably as a result of the low copy number of plasmid in these strains (see Fig. 4).

DNA of the 'recovered' pJDB219 was isolated from eight *E. coli* transformants selected by transformation to *leu*⁺ and tetracycline resistance, and from two transformants selected for tetracycline resistance alone. The *Eco*RI digestion pattern of seven of these plasmid isolates was identical to that of pJDB219 DNA. Three of the recovered plasmids contained the yeast DNA sequence in the type A rather than the type B configuration. Since pJDB219 DNA used to transform yeast was in only one configuration (type B) this confirms the theory that recombination occurs in yeast between the inverted repeat sequences of the yeast plasmid. It can also be concluded that the yeast DNA sequence in pJDB219 complements the *leuB* mutation in *E. coli* regardless of the orientation of this sequence with respect to the pMB9 sequence (the inverted repeat sequences separate pMB9 from the *leu*-complementing sequence).

The results presented in this article show that *S. cerevisiae* can be readily transformed by the hybrid plasmids and that despite the generation of unexpected recombinant plasmids in some clones the unaltered plasmid can be isolated from the yeast clones directly, or purified by recloning in *E. coli* and prepared more easily from bacterial cultures.

The inheritance of the *Leu*⁺ phenotype in the yeast clones containing pJDB219 or pJDB248 has been shown by tetrad analysis to be cytoplasmic (J. D. B. and Nasmyth, K., in preparation).

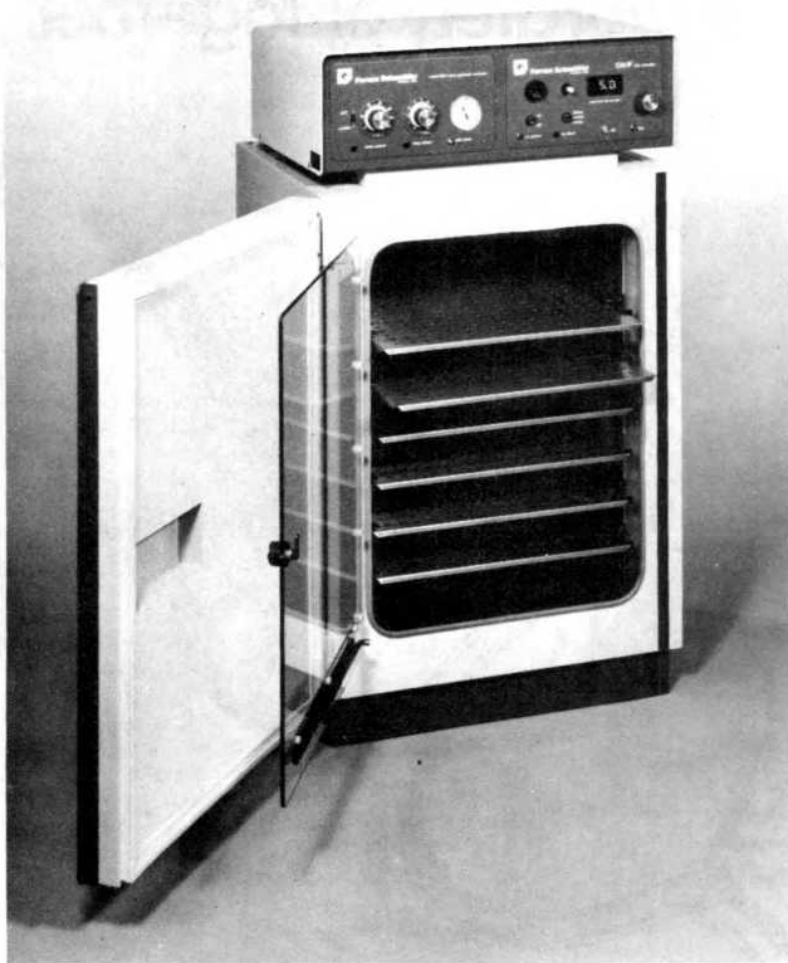
Yeast is a desirable organism to use in molecular cloning. It is readily and rapidly propagated. Its biochemical and physiological similarity to bacteria allows the use of techniques developed for bacterial systems. It offers the advantage to genetic studies of vegetative growth in both the haploid and the diploid states. The high frequency of transformation obtained with the system described here makes it feasible to isolate directly in yeast any yeast DNA sequence from a pool of hybrid plasmids carrying fragments representing the entire yeast genome.

All experiments using organisms containing recombinant plasmids were carried out under category I containment as recommended by the UK Genetic Manipulation Advisory Group. I thank Professor K. Murray and Dr R. B. Flavell for laboratory facilities and many materials, and J. F. Atkins and J. R. Bedbrook for discussions. Most enzymes were provided by K. Murray and J. R. Bedbrook. Calf thymus terminal transferase was a gift from the Corporate Lab., ICI. The work was supported in part by an SRC grant. An EMBO Short Term Fellowship was awarded to develop techniques for regenerating yeast protoplasts in the laboratory of Dr P. P. Slonimski. J. D. B. is a Beit Memorial Research Fellow.

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letters to nature

Atmospheric gravity waves generated during a solar eclipse

DURING a solar eclipse, the Moon's cool shadow, moving with supersonic speed through the Earth's atmosphere, should generate bow waves. In the experiments described here four microbarographs were used in an attempt to detect atmospheric pressure oscillations that were expected to accompany bow waves associated with the total solar eclipse of 23 October 1976. These internal gravity waves were found to have a peak-to-peak amplitude of 0.1 to 0.2 Pa, a period of 23 min and a velocity of $3.1 \times 10^2 \text{ m s}^{-1}$.

Figure 1 shows, in relation to the central line of the eclipse, the locations of the microbarographs at Tantanoola (T), Whyalla (W), Adelaide (A) and Kambalda (K), some hundreds to thousands of km apart. Their geographical coordinates are listed in Table 1. Four microbarograph records, sampled at 1-min intervals, are shown in Fig. 2. They all have dominant pressure oscillations at periods of the order of 20-25 min, with peak-to-peak amplitudes of 0.1-0.2 Pa. (Note that the microbarographs had broadband characteristics. Their maximum amplitude response was adjusted to be at a period of 6 min, which was approximately double their response to a 23-min period. It indicates that if other dominant periods had been present, these would also have been recorded.)

A microbarograph was installed at Kambalda (Western Australia) in anticipation that focusing of the bow-wave might occur in this locality due to curvature of the eclipse path and of the Earth's surface. In that case, a considerably greater amplitude would be expected at K compared with amplitudes at T, A and W which were well outside the expected focusing region. However, the present observations indicate similar amplitudes at all four stations, and give no suggestions of a focusing effect near K. The computation predicting focusing at K was subsequently found to be in error; S. Ball (personal communication) has shown that focusing of the bow wave should, in fact, have occurred at high northern latitudes.

At T, W and A, the weather remained fine throughout the recording time. At K, storms, accompanied by pressure fluctuations of 0.5-1.0 Pa amplitude, masked any bow wave recording except for the 2 h shown in Fig. 2.

To obtain a better estimate of the periodicity, a spectral analysis was made of each of the four records in Fig. 2. In every case, a period between 22 and 24 min was identified, the mean period being 22.7 min. This was the only periodicity common to all four records. It therefore seemed reasonable to attribute a 23-min period to a disturbance sufficiently widespread to affect all four stations, such as that generated by the eclipse.

As a further check that the 23-min period was associated with the eclipse, a comparison was made of the data shown in Fig. 3, namely the spectral components for T, which were: (1) derived for the nearly 5 h data in Fig. 2, including the eclipse interval and shortly afterwards; (2) derived for 83 min immediately before;

and (3) for 112 min immediately after the data in (1). (T was the only station for which data were available to make such a comparison.) The predominance of the 23-min period during and shortly after the eclipse, as indicated in (1), and its absence at adjacent times, confirms its association with the eclipse.

The present observation of 23 min is similar to the periodicity of about 20 min recorded^{4,5} in upper atmospheric disturbances after the eclipse of 7 March 1970.

An internal gravity wave of period 23 min should have a velocity⁶ of $3.1 \times 10^2 \text{ m s}^{-1}$. From this, and the known velocity of the eclipse 'point' along the central line of the eclipse path⁷, the positions of Q, T, S and R (called disturbance points) on the central line were estimated and are shown in Fig. 1. An eclipse-generated disturbance initiated at these points would arrive first at station K and then at T, A and W, respectively, before disturbance initiated elsewhere on the line. From the known times⁷ when the eclipse point moved through Q, R, S and T, an estimate was made of the times when the same phase of a bow wave should be recorded at K, W, A and T, respectively.

Table 1 Geographical coordinates of the four microbarographs

	Tantanoola (T)	Adelaide (A)	Whyalla (W)	Kambalda (K)
Latitude (°S)	37.7	34.8	33.0	31.2
Longitude (°E)	140.6	138.6	137.5	121.7
'Disturbance point' on central line of eclipse	T	S	R	Q
Comparative time (min) when eclipse was at dis- turbance point	20.5	17.75	15.5	0

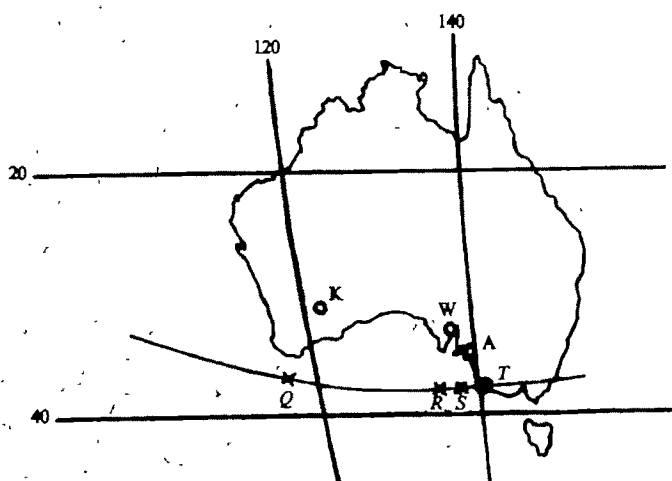


Fig. 1 Locations of the four microbarograph recording stations (T, A, W and K) and the central line of the eclipse.

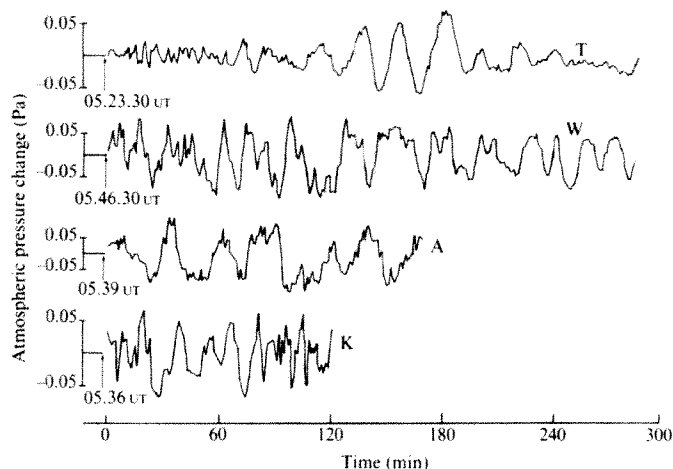
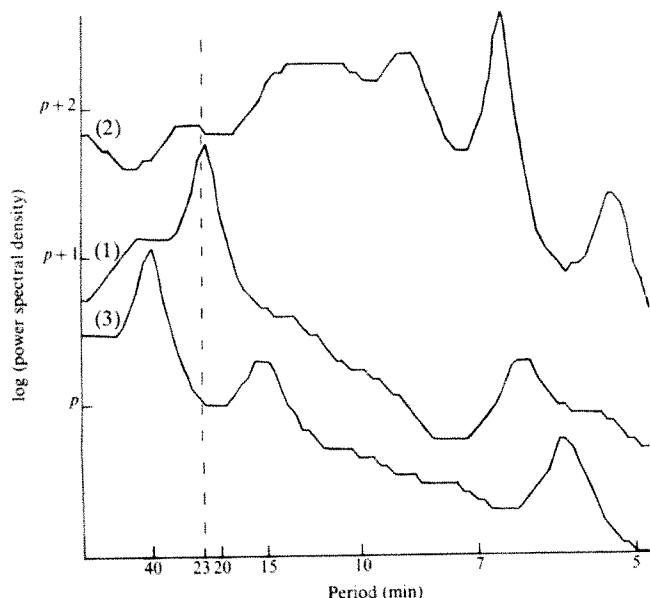


Fig. 2 Atmospheric pressure fluctuations recorded during and shortly after the eclipse at T, A, W and K.

The data were analysed further to confirm that the bow wave had the assumed velocity of $3.1 \times 10^2 \text{ m s}^{-1}$, and so to verify that an internal gravity wave was generated along the eclipse path. In the analysis, data from all stations were limited to the first 2 h plotted in Fig. 2, because useful K data were only available at that time. In estimating the bow wave velocity, it was convenient to imagine that a disturbance left Q, R, S and T simultaneously. To allow for the non-infinite eclipse velocity, an appropriate amount of time (indicated in the last row of Table 1) was subtracted from the actual times of recording for each station. The time difference between records from two stations, corrected in this way, is denoted t' . The interval, t' , between the occurrence of a particular phase of a disturbance at one station, and its occurrence at another station, is the time for a wave to travel between those stations. Knowing also the distance the wave has travelled, its velocity may be determined.

We wanted to estimate as accurately as possible the phase difference between each of the six pairs of records. To do this, cross-correlation coefficients, with time slips, were calculated for each pair of records. In Fig. 4, the cross-correlation coefficient, r , is plotted as a function of t' for the six pairs of records. (As an indication of the reliability of the data, note that the largest maximum in each of the TW and WK curves in Fig. 4 is statistically significant at the 5% significance level.)

Fig. 3 Spectral components of Tantanoola data recorded: (1) during and shortly after the eclipse; (2) immediately before data in (1); and (3) immediately after data in (1).



In principle, the value of t' at a particular correlation maximum for a pair of stations should be the time of travel of a bow wave from one station to the other, corresponding to the assumed velocity of $3.1 \times 10^2 \text{ m s}^{-1}$ ($=18.6 \text{ km min}^{-1}$). (Each curve in Fig. 4 has, in fact, several correlation peaks at approximately 23-min intervals, as expected. An acceptable correlation maximum would correspond to t' , giving a velocity of approximately the expected value.) However, the appreciable noise creates difficulty in accurately specifying the value of t' appropriate to a correlation maximum. To improve the accuracy, a sine curve of 23 min period was fitted to each cross-correlation curve. The arrows in Fig. 4 indicate when the sine curve giving best fit was a maximum, and these were taken to represent the correlation curve maxima.

The ordinate in Fig. 4 is d , the distance travelled by the bow wave from the first-mentioned station to the second. The six circles in Fig. 4 represent values of d as a function of t' , corresponding to correlation maxima. The circles for the station pairs AW, WK and AK lie close to a line of slope 18.6 km min^{-1} which passes through the origin. The observed time delays for those station pairs therefore affirm the assumed value for the bow wave velocity. The circles for the station pairs TA, TW and TK may be represented by a line of similar slope, but passing through $d = 0$ at $t' = -11 \text{ min}$. It seems significant that station T, on the central line of the eclipse, is included in all three of these latter station pairs. It suggests that the assumption of an atmospheric disturbance that occurred only on the central line of the eclipse is inadequate. In fact, the relevant width of the eclipse path was 174 km.

A cross-correlation analysis was carried out on the total T and W data in Fig. 2, extending over nearly 5 h. This gave a correlation curve maximum at $t' = 26 \text{ min}$, indicated in Fig. 4 by a square which lies close to the line of slope 18.6 km min^{-1} , passing through the origin. In contrast, the TW cross-correlation curve in Fig. 4, computed for only the first 2 h data, has a correlation maximum 11 min earlier.

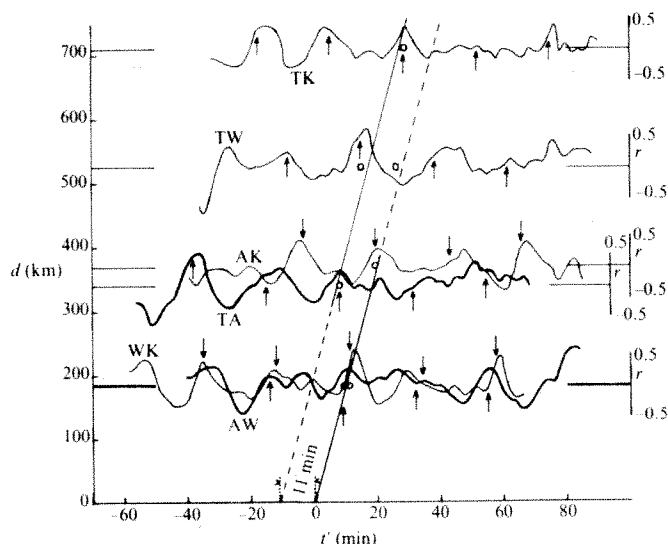


Fig. 4 Cross-correlation coefficients (r), with time slips, are shown for the six pairs of stations. t' is the time slip of the first-mentioned station of a pair ahead of the second; t' has been 'corrected' to allow for the non-infinite eclipse velocity. d is the distance travelled by a bow wave between the two stations of each pair.

Both sets of points in Fig. 4 can be represented by lines of slope 18.6 km min^{-1} , which affirms that the observed velocity was as expected for an internal gravity wave of period 23 min.

An apparent discrepancy is the additional 11 min delay observed in the early phase of the bow wave received at T, compared with that received at A, W and K. This may be explained in terms of an initial disturbance that occurred north

of the central line of the eclipse, and travelled a longer distance to T and shorter distances to A, W and K than would have been the case for a disturbance on the central line. The difference in travel times, being 11 min, would locate the actual disturbance source at, or near, the total eclipse boundary. As the data in Fig. 4 are accurate to ± 2 min, the observation could imply a source location somewhere from ground level to stratospheric heights. The point arising in the present context is that the apparent 11-min discrepancy is explainable, and that the data which involve station T are not inconsistent with other data in affirming the expected internal gravity bow wave velocity.

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First direct measurement of the beaming of Jupiter's decametric radiation

ONE of the most important features of the jovian decametric radioemission comes from the geometry of the observed radiation. The emission occurrence probability appears as a well defined function of the respective positions of Jupiter, the observer and the innermost galilean satellite Io, exhibiting several major sources which correspond to narrow ranges in zenocentric observer's longitude L (the so-called central meridian longitude) and in the synodic orbital phase of Io, Φ . These statistically established directivity properties were defined by Warwick¹ and Alexander², who showed that the repetition of the distinguishable dynamic spectra associated with each source occurring in a longitude range of 20° , is attributable to a $\pm 10^\circ$ beaming cone into which the radiation escapes in the ecliptic plane. Dulk³ stated more precisely that the emission is received on the Earth when Io makes certain angles with the line of sight, and that the dynamic spectrum varies in shape and frequency range with the magnetic longitude of Io. The beaming in latitude is also important⁴: the $\pm 3.3^\circ$ variation of the zenocentric declination of the Earth is sufficient to lead to a 15° variation of the statistical apparent sub-Io longitude of the emission. But these conclusions, based on statistics and on the repeatability of similar dynamic spectra in equivalent geometrical configura-

tions of Jupiter, Io and the observer, are not decisive, as they were obtained from a single direction at a given time. We present here the first direct experimental proof of a narrow beaming of Jupiter radiation, using simultaneous observations in two different directions, during the French-Soviet space radio astronomy experiment Stereo-5.

In this experiment, the Sun was simultaneously tracked from the Earth, at Nançay Observatory, and from the Soviet spacecraft Mars 7, at the frequencies 30 and 60 MHz (ref. 5). The observations were carried out for up to two hours per day, when the Sun was near the meridian at Nançay, from August 1973 to May 1974. During this period, the spacecraft was fitted with two linear antennas perpendicular to the ecliptic plane, which also allowed us to observe Jupiter. At 30 MHz, the antenna was a full-wave dipole; taking into account its efficiency and the electrical perturbation due to the spacecraft body, its effective gain was about 4 dB in the direction of the Sun, and much less in other directions. The signals were preamplified and detected by two automatic gain-controlled receivers with 0.5 MHz bandwidth and logarithmic responses. Both outputs were digitised at 4 Hz, and data were compressed and recorded on board, before being transmitted by telemetry to the USSR and then back to France. At Nançay, the equipment consisted of two 10 dB Yagis nearly orientated towards the Sun, feeding receivers of the same kind as on the spacecraft but with a 10 Hz sampling rate.

The broadband decametric Jupiter patrol at Nançay⁶ used a linearly polarised, log-periodic antenna, with a 10 dB gain, and a swept frequency spectrograph, covering the 20–40 MHz frequency, with a 15 kHz bandwidth and a sweep rate generally close to 10 Hz; in addition, a 1 MHz multichannel spectrograph⁷, tunable in frequency, was centred at 30 MHz during the stereo periods.

We were able to compare unambiguously two pairs of simultaneous track periods, 23 March 1974 and 15 May 1974, each free of solar activity (Fig. 1). On the first day, Jupiter was recorded on the Earth and not on the probe, and vice versa on the second day. These two cases, both of which correspond to the high frequency jovian Early source, are synoptically described in Fig. 2. The configuration in the solar system is displayed in Fig. 3. Note that, when giving flux densities or times for the spacecraft, we consider what would be seen by an observer on the line Jupiter-spacecraft at a distance of Jupiter equal to the Jupiter-Earth distance. From solar burst studies, we know that the sensitivity of the Earth is about four times that on the spacecraft for a source in the direction of the antenna maximum response (neglecting any problem of polarisation). We did not expect to receive many Jupiter events from the spacecraft, owing to the amount of available observation time. At 30 MHz, it emits for only a small percentage of time. It was also, for several weeks, pointing in directions of very low spacecraft antenna gain. Moreover, the solar activity often prevented any detection of the jovian emission. Hence we did not record Jupiter's activity when the stereo angle was small.

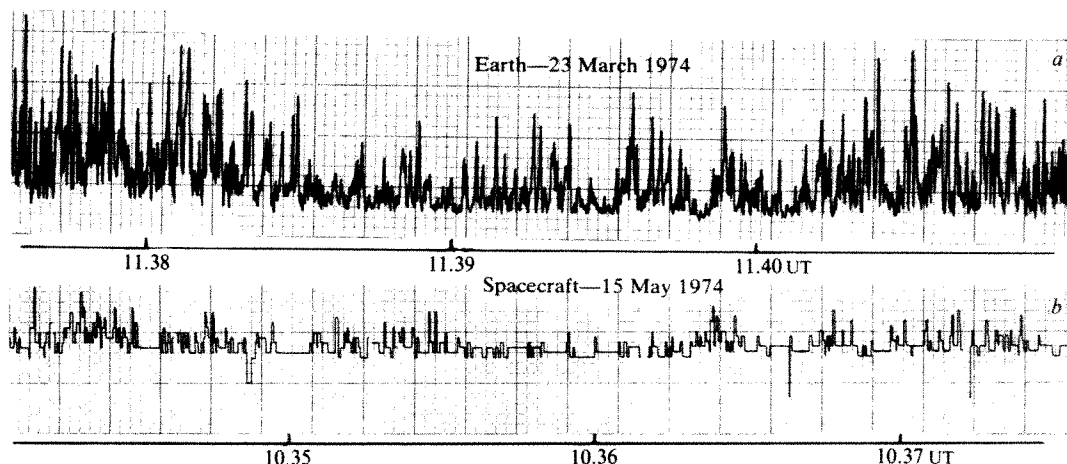


Fig. 1 Jupiter recorded at 30 MHz. On 23 March (a) the spacecraft is free of any activity; on 15 May (b) the Earth is free. The time scale for the probe is reset for a distance to Jupiter equal to that of the Earth at the same time.

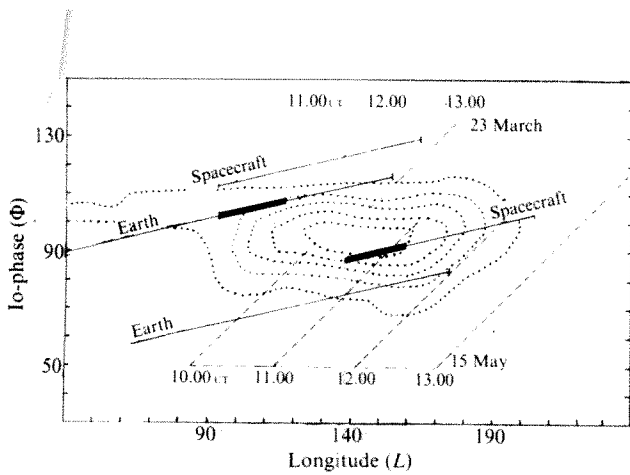
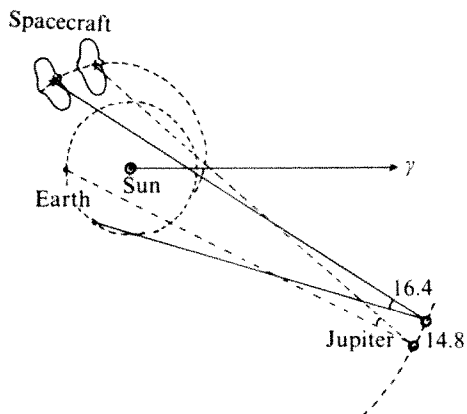


Fig. 2 Synoptic diagram of the Stereo observations of 23 March ($\theta = 14.8^\circ$) and 15 May ($\theta = 16.4^\circ$). Light lines correspond to the periods of observations; heavy lines sketch the observed jovian activity periods; dashes indicate, for both days, the UT on Earth and on the spacecraft, taking into account the delay due to propagation; dotted lines are the occurrence probability contours, as drawn from 7 to 41 MHz (University of Colorado's data).

Nevertheless, all the features of the emission recorded by the spacecraft on 15 May 1974 are typical of Jupiter activity. The emission is absent at 60 MHz and present at 30 MHz, where it reaches a maximum flux density about $8 \times 10^{-21} \text{ W m}^{-2} \text{ Hz}^{-1}$. It was recorded for half an hour; it consisted of a succession of bursts, which look like the well known L -bursts, with a pseudo period of the order of 2 min, exhibiting strong intensity fluctuations on a very short time scale (the solar bursts never have so short a time scale). These fluctuations might correspond either to the interplanetary scintillation or to jovian S -bursts, averaged by the time detection constant of the spacecraft receiver. Of course, the slower modulation (L -bursts) is not an ionospheric effect, as is usually assumed from the ground, but is characteristic of the emission itself and probably corresponds to drifting spectral structures in the time-frequency plane.

At that time, in Nançay, Jupiter was not recorded either by the 30 MHz receivers or at any frequency between 25 and 38 MHz by the swept frequency receiver. At the end of the observation period, the planet was at a very large zenith angle in Nançay; but it was also tracked by the University of Colorado's spectrograph, between 7 and 41 MHz, and no emission was observed⁸ from 09.00 UT until 12.00 UT, above the sensitivity threshold $5 \times 10^{-22} \text{ W m}^{-2} \text{ Hz}^{-1}$. We can estimate that, during the whole ground observation period, there was no emission more intense than one-tenth of the intensity recorded on the spacecraft. This is to be related to the Jupiter stereo angle $\theta = 16.4^\circ$ (that is, the Earth-Jupiter-spacecraft angle), at this date.

Fig. 3 Projection on the ecliptic plane of the positions of the spacecraft, the Earth and Jupiter on 23 March (dashes) and 15 May (solid line). The radiation pattern of the spacecraft is indicated.



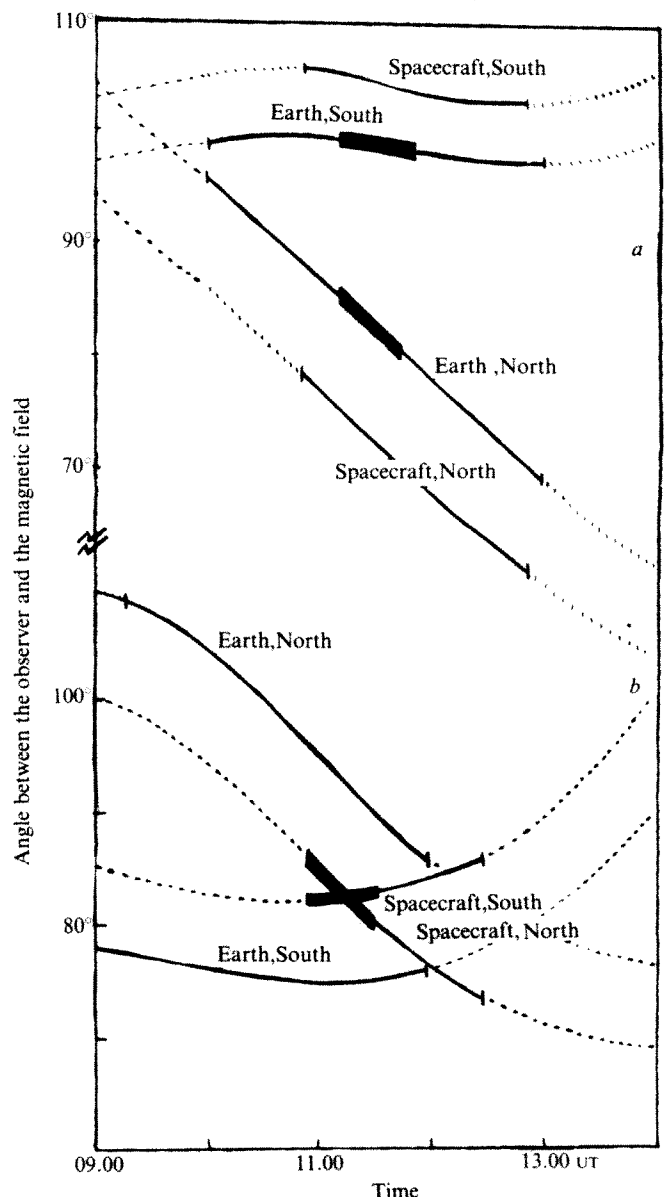
On 23 March 1974, Nançay recorded a strong Jupiter event, lasting 40 min, which reached about $3 \times 10^{-21} \text{ W m}^{-2} \text{ Hz}^{-1}$. It was visible on all the receiver outputs and showed rapid spectral structures. Aboard the spacecraft, whose sensitivity threshold was $\sim 10^{-21} \text{ W m}^{-2} \text{ Hz}^{-1}$, that is, one-third of the activity peak on the ground, no emission was recorded in the whole observation period. The stereo angle was $\theta = 14.8^\circ$.

Thus, both observations are the first direct evidence of the strong beaming of the decametric jovian emission: the recorded intensities, at the same instant, in two directions making an angle of 15° or 16° in longitude (and 0.2° or 0.4° in latitude) as viewed from Jupiter, differ by 5 or 10 dB at least.

Figure 2 shows in L - Φ coordinates the lines corresponding to the observation and emission periods, for the Earth and the spacecraft for the two days. The periods without emission correspond to regions of lesser occurrence probability.

In both cases, the spacecraft and the Earth swept the same ranges of longitude L , where the emissions occurred, whereas the ranges of Io-phase Φ were different. That seems to indicate that it is the 11° difference in Io-phase which causes the lack of correlation in the observations. Moreover, if we allow some possible variation in L -coordinate for the emission visibility, the

Fig. 4 Time variation of the departure angle between the observer and the magnetic field at the feet of the Io threaded magnetic flux tube; light lines, observation periods; heavy lines, emission periods. a, 23 March; b, 15 May.



Io-phase discrepancy was only 6° and 4° , respectively. Our observations may then be interpreted as an indication that the more rigorous angular condition comes from the position of Io on its orbit with regard to the observer. To illustrate this, supposing that the source of emission was at the northern or southern foot of the Io threaded flux tube (a generally accepted idea), we have computed (Fig. 4) the departure angle between the local magnetic field and the observer, using the D_4 -dipole model of Smith *et al.*⁹. On 23 March, this angle varies between 85° and 79° in the Northern Hemisphere (or 99° – 98° in the Southern) when the emission occurs on the Earth: aboard the spacecraft, during the observation period, the range was 78° – 61° (or 105° – 102°); on 15 May, the angle varies between 86° and 80° in the Northern Hemisphere (or 82° – 83° in the Southern) when the emission occurs on the spacecraft: on the Earth, the observed range was 110° – 86° (or 80° – 75°). Note that this angle differs by 9° at a given time between the Earth and the spacecraft and that, in any case, the ranges of variation overlapped. We conclude, therefore, that the decametric radiation should be emitted in a very narrow pencil beam, perhaps as narrow as a few degrees, which seems difficult to explain in terms of emission mechanism. We could imagine some strong propagation effects able to distort a less directive beam of emission.

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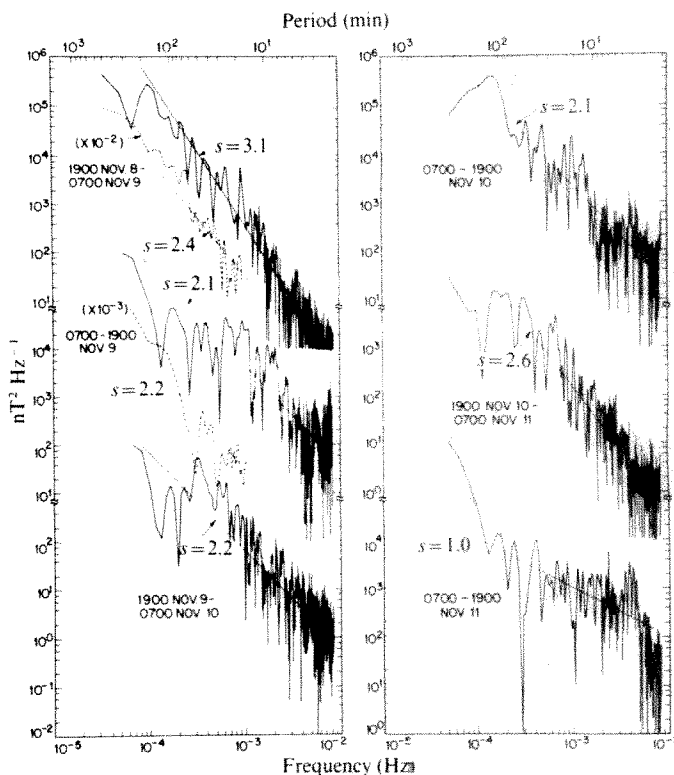
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Oscillations of the Sun and the geomagnetic field

THE suggestion by Toth¹ that the reported long-period oscillations of the solar atmosphere^{2,3} may be observed as oscillations of the geomagnetic field is an intriguing idea. It was suggested¹ that the ~ 2 h 40 min oscillations found in geomagnetic field data recorded at Tihany, Hungary, could reflect, through one of two possible mechanisms, similar period solar oscillations. In the first the geomagnetic field might have natural oscillations at this frequency that could produce environmental changes in the atmosphere^{4,5} and/or ionosphere and/or magnetosphere that would cause the ground-based telescope observations to reach erroneous conclusions. In the second mechanism the oscillations in the solar atmosphere might produce similar frequency oscillations in the interplanetary magnetic field which, through coupling with the geomagnetic field at 1 AU, would cause similar period variations in the field at the Earth's surface. Such oscillations would be in the range of periods of solar wind fluctuations measured near Earth and often attributed to Alfvén waves⁶. Regular, periodic variations in the interplanetary magnetic field with a

Fig. 1 Power spectra of six consecutive sets of 12 h of geomagnetic fluctuation data recorded at Durham, New Hampshire in 1975 (solid lines). The times and dates indicated for the spectra correspond to the local time of the station (LT $\sim$ UT $- 5$ h). The slope of the power-law fit through each spectrum is drawn. Dotted lines represent the low frequency portions of two spectra from Siple Station, Antarctica.



period near that of the reported solar oscillations have not been reported. We have spectral analysed geomagnetic field data on the same days as those published by Toth¹ using data from our fluxgate magnetometer stations in New Hampshire and in Quebec and data from the conjugate area at Siple Station, Antarctica⁷.

Our lowest latitude station (Durham, New Hampshire) is at the geomagnetic shell parameter $L \sim 3.2$; the highest latitude station (Girardville, Quebec) is at $L \sim 4.4$; Siple Station is at $L \sim 4.1$. Local time (LT) of our stations is $\sim$ UT $- 5$ h. The three components of the magnetic field are sampled at two second intervals and written digitally on magnetic tape. The instruments' noise levels are ~ 0.2 nT; the digitisation increment is 0.06 nT. We used our standard analysis method: the fast Fourier transform with a Thomson data window⁸ applied in the time domain. The geomagnetic observatory used by Toth in his analysis is located at $L \sim 1.9$, approximately 6 h east of the longitude of our stations.

Several spectra (12-h intervals) from Durham for 9–11 November 1975, the days reported by Toth¹, are shown in Fig. 1. Also shown (dotted lines) are the low frequency portions of Siple spectra for two of the time intervals. A power law frequency dependence $P(f) \propto f^{-s}$ was fit through each spectrum. The fits are shown superimposed on the spectra in the figures and the values of s for each spectrum are indicated. In none of these spectra do we find any evidence for a significant peak at a period of ~ 2 h 40 min. Because we find no significant spectral peaks in either the Northern Hemisphere data or in the conjugate (Siple) data, we can rule out a global magnetospheric phenomenon, driven either internally or externally, as the cause of the reported magnetic variations¹.

The data analysed here are expressed in power per unit frequency. At 10^{-4} Hz, the power level 10^5 nT² Hz⁻¹ corresponds to an amplitude of ~ 4 nT in a bandwidth of 2×10^{-4} Hz, an amplitude sensitivity comparable to the amplitude spectrum of Toth¹.

A relationship between the reported long period variations of the solar atmosphere and the geomagnetic field would have important implications for interplanetary field/geomagnetic field coupling, as well as for the solar wind flow in the inner heliosphere. However, we find no evidence of solar oscillations being reflected in the Earth's magnetic field on a global scale. The reported geomagnetic variations with a period of ~ 2 h 40 min must be local, related to geophysical sources in the vicinity of the observing station. We note a recent report⁹ that the envelope of modulation of geomagnetic pulsations in the period range 10–45 s as well as the auroral electrojet index AE had periods of ~ 2 h 40 min, possibly related to the solar oscillations.

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Large baryon densities attainable in high energy nuclear collisions

NUCLEAR densities at least four and as much as 10 times normal should be achieved in U–U collisions with centre-of-mass kinetic energies between 1 and 4 GeV per nucleon, which would be much higher than previous discussions have indicated. Such collisions would give the best opportunity of making a stable or long-lived phase of high baryon density. It is suggested here that the accelerators at CERN would be well suited to explore the energy range of interest, both with fixed targets and with colliding beams.

Nuclear physics has traditionally focused on the properties of nuclear matter in or near 'normal' conditions, meaning those prevailing in known nuclei. The properties of systems with very high baryon density are unknown, although there have been interesting speculations about stable or nearly stable high density phases^{1–6}. In the absence of spontaneous transitions to such phases¹ (which might well be inhibited by enormous barriers) the only known way to produce high baryon density is to bombard heavy nuclear targets with heavy nuclear projectiles⁷.

Early theoretical discussions of such reactions were restricted to the nonrelativistic domain, with centre of mass (c.m.) kinetic energies much less than rest energies of the colliding nuclei. Density enhancements were computed by treating the nuclei as colliding drops of 'nuclear fluid', forming a shock front where they meet. These assumptions are valid for c.m. energies less than about 0.25 GeV per nucleon, at which the shock 'front' becomes as thick as the nuclei themselves, and a density less than four times normal is found for a plausible nuclear equation of state⁸.

The limitations on the shock wave approach suggested that higher energies could not generate still higher compressions. However, recent computations with a two-fluid model, in which bits of fluid at high relative velocity are coupled only by a frictional drag, indicated that high density could be attained during mutual interpenetration and slowing of target and projectile without shock formation⁹. An attempt to understand these results of complex numerical calculations has led to the following simple considerations, which assume minimum

compressibility and therefore give lower limits on the density enhancements to be expected at relativistic c.m. energies.

Uranium is a sufficiently large nucleus so that, at a 10–30% level of accuracy, a nearly head-on collision (≈ 0.1 b out of ≈ 7 b total reaction cross section) may be described as the meeting of two slabs 15 fm thick with nucleon density about 0.15 fm^{-3} . Of course, in the c.m. frame each slab would be Lorentz contracted, with density increased and thickness decreased by the Lorentz factor $\gamma = W/2M$, where W is the total c.m. energy and M is the mass of a uranium nucleus. Suppose that for a given γ each slab is thick enough to stop (in the c.m. frame) a nucleon in the other slab. Then the combined system will eventually be confined to a thickness less than $15/\gamma$ fm, because the back of either slab (assumed to have relativistic velocity) must progress at least halfway to the meeting plane before even a light signal from that plane could reach it. Consequently, at one moment the average baryon density will be at least 2γ times normal.

To get the maximum γ for stopping, note that a 40 mb nucleon–nucleon cross section corresponds to an average of nine interactions on crossing the slab. This number would be increased if repulsive correlations among the target nucleons were taken into account, but would be lower near the edges of the actual finite spheroidal nuclei. Suppose that the interaction probability is unchanged by previous collisions exciting the nucleon. A single high-energy nucleon–nucleon collision leaves a nearly uniform distribution in the fraction, x , of projectile momentum retained by a fast final nucleon¹⁰. If successive collisions had the same property, as they would for widely spaced target nucleons, then after N collisions the mean value of x would be $(N+1)^{-1}$, so that, converting to the c.m. frame, the stopping γ would be $1/2\bar{x} = (N+1)/2$, and the density enhancement would be greater than $N+1 = 10$ in our example. Barring an unexpected collective interaction between projectile and target, this estimate gives the upper limit on the stopping γ . A safe lower limit is $\gamma = 2$: at this γ , the target is contracted by a factor 7 in the projectile frame, giving a geometrical thickness just over 2 fm, so that in all relevant frames of observation there would be at least three collisions, each separated from the others by a time interval >1 fm (taking $C \approx 1$). This should be long enough for collisions to be independent, so that the effective N is at least 3, giving a stopping γ of 2 and making this a self-consistent lower limit. An indication of the most likely γ should come from analysing spectra of fast nucleons emitted in high energy nucleon–nucleus collisions. Such an experiment is underway at CERN (B. Povh, personal communication).

Farley¹¹ stated that the c.m. energy range 1–10 GeV per nucleon is important. As shown here, 1–10 GeV (rather than values around 0.25 GeV suggested by older work⁸) is the range in which one would expect to see a transition from stopping to transparency, or passage of the two nuclei through each other with some excitation and deceleration. If stopping were still found at 10 GeV per nucleon, that would be a remarkable discovery of a new collective effect.

From these arguments, CERN is a logical site for exploration of the high-baryon-density frontier. Uranium ions injected into the PS could be used in all three major installations at CERN (PS, SPS, ISR) for fixed target or colliding beam experiments in the c.m. energy range 1–10 GeV per nucleon. From the technical point of view, because low energy (MeV per nucleon) partly stripped uranium beams have been made at GSI in Darmstadt, the main new development would be acceleration to energies of a few hundred MeV per nucleon and full stripping of the ions. Studies for the Berkeley Bevalac indicate that this could be accomplished by substantially improving the vacuum of the booster which feeds the PS, with little if any modification in the PS itself (H. A. Grunde, personal communication). (A plan to generate 1 GeV per nucleon uranium at the Bevalac is expected to cost \$5 million, so that an effort on a similar scale at CERN would have a capital cost roughly 1% of the annual budget¹².)

Many questions remain about observation, interpretation and possible application of phenomena associated with high baryon

density. The central point is that an almost model-independent argument shows high density can be produced, and from previous experience, explorers of such a new domain may find rich rewards—perhaps even stable dense matter.

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Photoelectrochemical reduction of aqueous carbon dioxide on p-type gallium phosphide in liquid junction solar cells

THE non-biological reduction of carbon dioxide to organic compounds is of interest, as an alternative to natural photosynthesis, for the production of organic raw materials or fuel. In one approach, the required energy was supplied by irradiation with UV light, in the presence of ferrous salts, and resulted in the production of formic acid and of formaldehyde¹. In another approach, the energy was supplied from an external power source by electrochemical reduction of aqueous carbon dioxide. The reduction of carbon dioxide and production of formic acid during the electrolysis of sodium bicarbonate in aqueous solutions has also been reported², and a study of the reduction of carbon dioxide on a mercury cathode reviews earlier work³. Polarographic measurements on mercury electrodes showed that carbon dioxide, rather than the bicarbonate ion, is the electroactive species, with a half-wave reduction potential of -2.1 V (relative to SCE), and that formic acid is the only product⁴. We report here the photoassisted electrolytic reduction of aqueous carbon dioxide, achieved using p-type gallium phosphide as a photocathode, with part or all of the energy being supplied by light. The products were formic acid, formaldehyde and methanol.

Semiconducting solids have been intensively studied for their interaction with electrolytic solutions, and the theory of interaction of illuminated semiconductors with electrolytes has been reviewed⁵. In photoelectrochemical cells, the semiconductor electrode immersed in the electrolyte is connected through an external circuit to a counterelectrode. In p-type semiconductors in the dark, there are many holes in the valence band, but only a small number of electrons in the conduction band. Illumination by light more energetic than the bandgap increases the number of electrons in the conduction band. This increase in electron density in the conduction band results in a cathodic current⁷. Electron transfer to a suitable acceptor in the solution reduces this acceptor.

The photoassisted electrolysis of water to hydrogen and oxygen has been carried out using either p- or n-type semiconductors under illumination, with metal counterelectrodes as anodes or cathodes, respectively⁷⁻¹⁰. In these cases, an external electrical bias potential was needed to drive the reaction. The use of combinations of n- and p-type semiconductors such as n-TiO₂ and p-GaP, and the illumination of both electrodes, resulted in 'self-driven' photoelectrolysis cells not requiring an external bias¹¹.

The photoelectrochemical reaction system used in the present work consisted of a closed double-walled borosilicate glass beaker (about 30 ml capacity), with thermostat set at 25 °C, containing an aqueous buffer solution as electrolyte, into which dipped a photoelectrode consisting of a single crystal of p-gallium phosphide (Zn doped; resistivity 0.19 Ω cm; front surface area 0.24 cm²; etched for 1 min in concentrated HNO₃-concentrated HF-H₂O, 3:1:4 v/v; the ohmic contact to the back surface of the crystal was made by an indium-zinc solder. The back surface and the external lead were insulated using an epoxy cement). The counterelectrode was a carbon rod (6 mm diameter), and a saturated calomel electrode served as a reference. Carbon was chosen as the counterelectrode, as it was reported that formic acid and other C, H, O compounds were not oxidised on carbon electrodes². In some experiments, p-GaAs and p-Si were tested as photocathodes, but n-TiO₂ and n-Si were used as photoanodes. A light-beam from a stabilised high-pressure mercury arc or from a projector halogen lamp was focused with the help of a condensing lens on to the GaP crystal. Oxygen-free carbon dioxide (purified by passing through an aqueous solution of vanadous chloride) was continuously bubbled through the electrolyte. A side-port fitted with a rubber septum and syringe served to withdraw samples without opening the flask.

Using the above cell combination, both in the dark and under illumination, a measurable current through the external circuit could be obtained only by applying a cathodic bias to the p-GaP electrode (Fig. 1a). With the application of a cathodic bias of -1.0 V (relative to SCE) to the p-GaP, the initial photocurrent density was 6 mA cm⁻², whereas the dark current density was only 0.1 mA cm⁻². The photocurrent decreased gradually, but after 24 h stabilised at about 1 mA cm⁻².

Analysis of the electrolyte solution showed the presence of formic acid (analysed by reduction with magnesium and chromotropic acid assay¹³, as well as by alkaline permanganate titration¹⁴), formaldehyde (chromotropic acid assay), and methanol (gas chromatography on Poropak Q). After 18 and 90 h of irradiation, the concentrations of formic acid produced were 1.2×10^{-2} and 5×10^{-2} M, those of formaldehyde 3.2×10^{-4} and 2.8×10^{-4} M, and those of methanol 1.1×10^{-4} and 8.1×10^{-4} M, respectively. Thus, in contrast to the reduction of carbon dioxide on metal cathodes, the photoelectrolysis on p-gallium phosphide does not stop with the production of formic acid, but proceeds further, yielding formaldehyde and methanol. The approximate constancy in concentration of formaldehyde may indicate that this product reaches a steady state of formation and further reaction.

The efficiency of conversion of radiant energy into the chemical energy (optical conversion efficiency) of the reduction products of carbon dioxide was calculated by adaptation of the formula used previously^{9,10} for the photoelectrolysis of water to hydrogen and oxygen (in which each product is assumed to be formed exclusively):

$$\text{Optical conversion efficiency} = 100 I_c [(\Delta H/Z) - V_B] / I_a$$

where I_c = current density (mA cm⁻²); I_a = incident light intensity (mW cm⁻²); ΔH = heat of combustion (= 2.962, 2.639, 5.915 and 7.259 eV for hydrogen, formic acid, formaldehyde and methanol, respectively); Z = number of electrodes required in the reduction of one molecule of carbon dioxide to a molecule of product (= 2, 2, 4 and 6 for production of hydrogen, formic acid, formaldehyde and methanol, respectively); V_B = electrical bias (V).

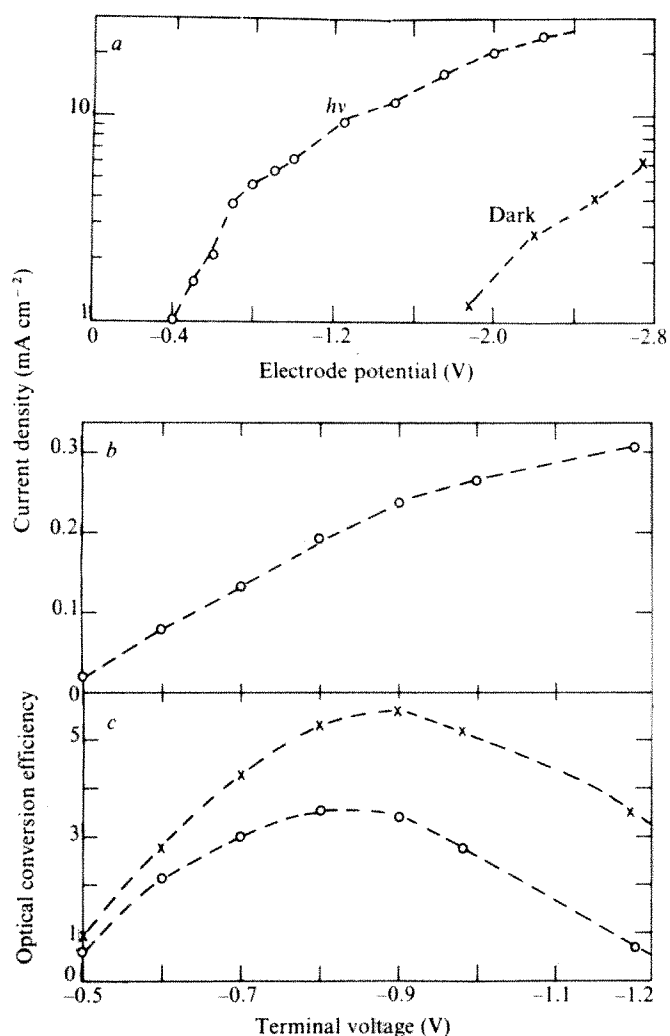


Fig. 1 *a*, Current density (log scale) as a function of p-GaP potential (relative to SCE) for the cell carbon/K₂HPO₄-KH₂PO₄, 0.05 M, pH 6.8, CO₂/p-GaP. Illumination, high-pressure Hg-lamp, unfiltered; 210 mW cm⁻². *b*, Current density as a function of terminal voltage for the cell carbon/K₂HPO₄-KH₂PO₄, 0.05 M, pH 6.8, CO₂/p-GaP. Illumination: halogen lamp, filtered to transmit 365 nm band; *I*_a 2.4 mW cm⁻². *c*, Optical conversion efficiency (%) as a function of terminal voltage for production of CH₂O (x) and CH₃OH (O) in the experiment shown in (*b*).

In an experiment using filtered light of 365 nm wavelength (Fig. 1*b*), the maximal optical conversion efficiencies for production of either formaldehyde or methanol were thus determined to occur at bias voltages of -0.8 to -0.9 V (Fig. 1*c*), and were 5.6 or 3.6%, respectively. As only 17.3% of solar irradiance (at AM2) is in the near UV and blue regions (315–510 nm)¹⁵, and thus effective for exciting p-gallium phosphide (bandgap 2.25 eV), the maximal solar energy conversion efficiency to formaldehyde or methanol is calculated to be 0.97 or 0.61%, respectively.

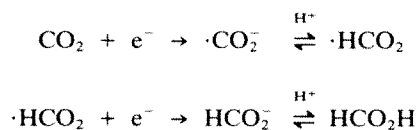
In another experiment, an n-type polycrystalline TiO₂ semiconductor was used instead of carbon as the counterelectrode, and as electrolyte a 0.1 M solution of lithium carbonate (30 ml). After 16 h of illumination (unfiltered light of Hg-lamp) on both electrodes, maintaining a constant current of 0.50 mA (current density 2.1 mA cm⁻² on the GaP cathode), and letting the negative bias on the p-GaP cathode rise gradually from -0.86 to -1.4 V (relative to SCE), the solution reached a concentration of 1.0 × 10⁻³ M methanol (or 3 × 10⁻⁵ mol). Considering that six electrons are required for the reduction of 1 mol of carbon dioxide to methanol, this corresponds to a current yield of 60%.

Using a commercial silicon solar cell (2 × 1 cm² area) as an electrode, connecting its p-junction through an external lead to

the p-GaP crystal, immersing both electrodes in 0.1 M Li₂CO₃, and illuminating the n-junction of the silicon as well as the p-GaP, no external electrical bias was required, as the two photoelectrodes produced a sufficient potential to drive the reaction. After 38 h of irradiation in the presence of carbon dioxide, the initial current of 0.05 mA had gradually decreased to 0.01 mA. During this period, the concentrations of formaldehyde and methanol reached 2 × 10⁻³ and 3 × 10⁻⁴ M, respectively.

Photoassisted reduction of aqueous carbon dioxide was also achieved using a p-type gallium arsenide single crystal electrode. However, in this case the cathodic bias required was such that no net gain in energy conversion was obtained.

The mechanism of cathodic reduction of carbon dioxide presumably involves stepwise electron transfer reactions. The first stable product, formic acid, must be due to a two-electron transfer. Possibly, the mechanism is similar to that proposed for the reduction of carbon dioxide on mercury cathodes¹⁶,



In contrast to the electrochemical reduction of carbon dioxide on metal cathodes, which stops essentially at this stage because of the high overpotential for formic acid reduction, in the case of the photoelectrochemical reaction on p-type semiconductors, the further reduction of formic acid occurs readily, producing formaldehyde and methanol.

Further development of the application of semiconducting solids to the photoelectrochemical reduction of carbon dioxide may provide a new approach to the production of organic raw materials and fuel.

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Characterisation of organic acids trapped in coals

THE characterisation of trapped organic compounds which occur in geological materials such as coal and oil shale can provide an insight into both the origin of the organic matter and its subsequent modification through chemical reactions. Much work has been done on the isolation and identification of polar and nonpolar compounds in carbonaceous materials^{1–7};

however, aromatic acids in coals have received very little attention. Here we detail the characterisation and distribution of aromatic acids extracted from lignite and bituminous coals.

Freshly ground raw lignite from Sheridan, Wyoming was first treated with 5% aqueous HCl at room temperature to free the acids from the minerals¹, and then extracted with 2.5% aqueous sodium hydroxide at 35 °C for 16 h. After work up by the usual method¹, soluble organic acids equal to 2.6% by weight of the lignite were obtained. In the same conditions, only 0.23% of acidic compounds were recovered from an Illinois no. 2 bituminous coal. No organic acids were isolated from a Pennsylvania anthracite coal. The elemental composition and description of these coals have been given elsewhere^{7,8}.

A fraction of the organic acids isolated from the lignite was derivatised with diazomethane for analysis. Another fraction was derivatised with d_6 -dimethylsulphate⁹ to yield tri-deuteriomethyl-labelled derivatives. The derivatives were analysed by gas chromatography-mass spectrometry (GCMS), solid probe MS and high resolution MS using techniques which have been described previously^{7,10}. A gas chromatogram of the mixture derivatised with diazomethane is shown in Fig. 1 with numbered peaks identified in Table 1. The distribution of the organic acids as methyl esters was determined by measuring areas of GC peaks with a correction for the effective carbon number for each compound (Table 2).

Note that, as shown in Table 2, the alkaline extractable acids are qualitatively similar to those obtained from selective oxidation of the macromolecular material of the same lignite coal with $\text{aq-Na}_2\text{Cr}_2\text{O}_7$, (refs 7, 8), H_2O_2 - CH_3COOH (refs 7, 8, 11) or 15% HNO_3 (ref. 8).

Of particular interest is the identification of large amounts of benzene di- and tri-carboxylic acids and their derivatives which have not been found in the mild alkaline extracts of shale^{12,13}, lignin¹⁴⁻¹⁷, peat¹⁸ or humic acid^{14-17,19}. No C_6 - C_3 carboxylic acids (benzene ring with C_3 side chain)^{15,20} were isolated from coals, whereas these acids are commonly found in the extracts of the above. Note also that the phenolic acids identified have only one hydroxy group but no methoxy group. This was determined from GCMS and HRMS of the sample derivatised with d_6 -dimethylsulphate. This contrasts with the presence of di-, polyhydroxy, or methoxy benzene derivatives in the alkaline extracts or degradation products of the geological materials described above. The alkaline extract of bituminous coal was found to consist mainly of benzene di-, tri- and tetra-carboxylic acids (31.7% of the extract) and their methyl derivatives (39.9%), and naphthalene mono- and di-carboxylic acids (9.8%). Phenolic acids (2.2%), methylfuran di-carboxylic acid (2.9%) and aliphatic dibasic acids (4.5%) were also identified in small amounts. The remaining 9% has not yet been identified. No C_6 - C_3 carboxylic acids or benzoic acid were detected. Benzene and naphthalene carboxylic acids have been identified as major

Table 1 Organic acids from lignite coal identified as their methyl esters by GC-MS and high resolution MS

Peak no.	Compound
1	Succinic acid
2	Glutaric acid
3	Methylfuranmonocarboxylic acid
4	Mixture of 3-methyl, 2,3-dimethyl and 3,3'-6,6'-tetramethyl dibasic acids
5	Benzoic acid
6	Methylbenzoic acid
7	Dimethylbenzoic acid
8	Methoxybenzoic acid (<i>m</i> - or <i>p</i> -)
9	<i>o</i> -Methoxybenzoic acid
10	Methoxymethylbenzoic acid
11	<i>o</i> -Methoxymethylbenzoic acid
12	1,2-Benzenedicarboxylic acid
13	1,4-Benzenedicarboxylic acid
14	1,3-Benzenedicarboxylic acid
15	Dimethoxydimethylbenzofuran (T)
16	Dimethylfurandicarboxylic acid
17	<i>o</i> -methylbenzenedicarboxylic acid
18	Methylbenzenedicarboxylic acid
19	Dimethylbenzenedicarboxylic acid
20	<i>o</i> -Methoxybenzenedicarboxylic acid
21	Methoxybenzenedicarboxylic acid
22	Methoxymethylbenzenedicarboxylic acid
23	1,2,4-Benzenetricarboxylic acid
24	1,2,3-Benzenetricarboxylic acid
25	1,3,5-Benzenetricarboxylic acid
26	Methylbenzenetricarboxylic acid
27	Methoxyphenyldihydrobenzofuran (T)
28	Mixture of compounds including methoxyphenyldihydrobenzofuran (T)
29	Dimethylbenzenetricarboxylic acid
30	<i>o</i> -Methoxybenzenetricarboxylic acid
31	Methoxybenzenetricarboxylic acid
32	Homologue of dehydroabietic acid (see text, T)
33	Methoxybenzyldihydrobenzofuran (T)
34	Methoxymethylphenyldihydrobenzofuran (T)
35	Dehydroabietic acid
36	Methoxymethyldibenzofuranmonocarboxylic acid (T)

T means that identification is tentative

products from the selective $\text{aq-Na}_2\text{Cr}_2\text{O}_7$ oxidation of the same coal^{7,8,21}. These facts may elucidate the reactions which occurred during the transformation of organic material from biopolymers (lignin, humic acid and so on) to geopolymers (coals). It is generally agreed^{1,15-17,22} that the essential reactions, including degradation of side chains, dehydrogenation, hydroxylation, demethylation, demethoxylation, cleavage of ring structures and polymerisation, occur during these transformations. Welte has suggested²² that at the stage of coal formation (early

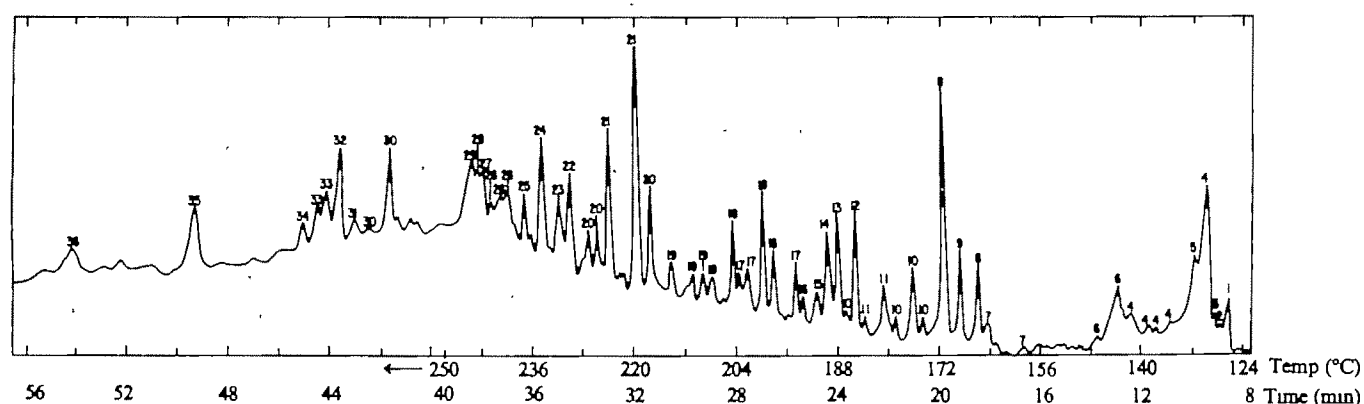


Fig. 1 Gas chromatogram of the methyl esters of the carboxylic acid fraction from lignite coal. The analysis was carried out on a Perkin-Elmer 3920B gas chromatograph interfaced to a modified Bendix model 12 time-of-flight mass spectrometer with a continually variable split between a flame ionisation detector and the source of the mass spectrometer. The separation was made on a $15.2 \text{ m} \times 0.51 \text{ mm}$ SCOT column coated with OV-17 and temperature programmed over $100\text{--}250^\circ\text{C}$ at 4°C min^{-1} .

Table 2 Organic acids identified in the alkaline extract and oxidation products of lignite coal

Acid groups	Alkaline extract (%)	Oxidation product (%)		
		aq-Na ₂ Cr ₂ O ₇ *	H ₂ O ₂ -CH ₃ COOH†	15% HNO ₃ ‡
Benzenecarboxylic acid	16.6	59.2	36.1	63.3
Methylbenzene carboxylic acid	16.0	4.3	7.6	13.9
Phenolic acid§	40.4	7.4	22.1	1.5
Furancarboxylic acid	0.8	—	15.9	3.2
Aliphatic dibasic acid	10.8	**	18.2	12.9
Terpenoid acid	6.6	—	—	—
Others	4.3	26.6¶	—	3.2
Unidentified	4.5	2.5	—	2.0

* 100% of coal was oxidised. 92% of total oxidation product is shown. The remaining 8% is humic acid-like material and nonacidic compounds.

† 80% of coal was oxidised. 50% of product is shown. The remaining 50% is humic acid-like material.

‡ 74% of coal was oxidised. 48% of product is shown. The remaining (52%) is humic acid-like material and nonacidic compounds.

§ Aromatic rings containing phenolic OH of lignite coal would have been destroyed by these oxidants except H₂O₂-CH₃COOH. The phenolic acid identified in these two oxidation products may be derived from aromatic rings containing methoxy group.

|| See peak nos 15, 27, 33 and 34 in Table 1.

¶ Carboxylic acids of naphthalene, phthalan, chroman, dibenzo-*p*-dioxin, xanthone, dibenzofuran and pyridine were identified (see refs 7 and 8).

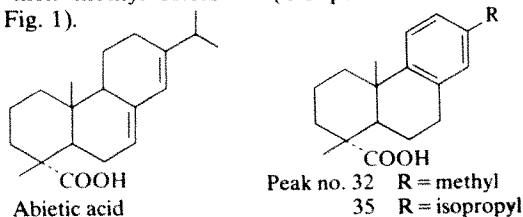
** Several acids were identified but not estimated.

—, not found.

katagenesis), functional groups are lost and CO₂ and water are produced in coals, and hydrocarbons are formed simultaneously. The benzene mono-carboxylic acid and its derivatives identified in the extracts are thought to be largely degradation residues from biopolymers at early stages of coalification, whereas the benzene di- and poly-carboxylic acids and their derivatives and naphthalene-carboxylic acids are produced in later stages by oxidative degradation. Perhaps, in the geological environment, oxidative cleavage of C-C bonds proceeded along the periphery of the coal macromolecules with rupture of bridging linkages between aromatic units. The presence of aliphatic dibasic acids in the extracts suggests that they are derived, in part, from these linkages. Indeed, oxidation of lignite and bituminous coals with various oxidants^{7,8,21,23} gave benzene di- and poly-carboxylic acids as the most abundant products; however, little or no benzene mono-carboxylic acid was found in the products.

An alkaline extract of shale was found to yield alkylbenzoic acids and phenylalkanoic acids which resemble their cyclic terpenoid precursors^{12,13}. On the other hand, no ethyl or longer-chain alkylaromatic acids, or phenylalkanoic acids were found in the present work which apparently indicates that coal has undergone more rigorous geological changes than shale. Therefore, the aromatic acids may have undergone dealkylation leaving more stable derivatives.

We have found two diterpenoid acids in the lignite extract, dehydroabietic acid and its homologue, that are apparently derived from the diterpenoid resin acid, abietic acid, which is commonly present in the resins of conifers¹⁻³. They were identified as their methyl esters^{24,25} (GC peaks 35 and 32 respectively, Fig. 1).



These two terpenoid acids are the probable precursors for related diterpenoid hydrocarbons which we have characterised in the benzene-methanol extract of this lignite⁷.

Note the presence of several furan, benzofuran and dibenzofuran acids extracted from the lignite with 2.5% aq-NaOH. These compounds may have been derived from lignans¹⁷ such as furoguaiacin and pinosresinol which are commonly found in heartwood, or as suggested by Flaig¹⁵, they might be degradation products from dibenzofuran derivatives which were formed from condensation reactions of phenolic compounds during the coalification.

In the transformation from lignite coal to bituminous and anthracite coals, the present work and our previous work on the trapped hydrocarbons in coals⁷ indicate that the following reactions probably occurred. (1) Extensive degradation of phenols; very few phenolic acids (2.2%) were found in the alkaline extract of bituminous coal, whereas the alkaline extract of lignite coal yielded 40.4% phenolic acids. (2) Loss of carboxyl group; the bituminous coal extract contained much less acids. No acids were found in the extract of anthracite coal. (3) Dehydrogenation and partial dealkylation of side chains; demonstrated by the increase in abundance of methyl aromatic hydrocarbons and a decrease in the amount of terpenoid type hydrocarbons in the organic solvent extract of bituminous coal as compared to lignite⁷.

Thus, the macromolecules in bituminous coal contain a greater proportion of aromatic structure and greater degree of crosslinking than is found in lignite. Benzene tetra-carboxylic acids and naphthalene-carboxylic acids were found in the extract of bituminous coal.

The organic acids extracted probably are both oxidative degradation products and residuals of the original coalification process. These acids, in particular the aromatic acids, were found to be characteristic of coals and different from those acids derived from biopolymers.

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Synthesis and structure of synthetic zeolite ZSM-11

ZSM-5 (refs 1, 2) is a member of a new class of shape selective catalysts which have unique channel structures with 10-membered ring openings. These catalysts have unusual properties. They can selectively convert methanol to high quality gasoline³⁻⁶ and are used in commercially significant processes, such as distillate dewaxing, ethylbenzene synthesis, xylene isomerisation and toluene disproportionation^{5,7}. The framework of ZSM-5 (ref. 2) contains a novel configuration of linked tetrahedra consisting of eight five-membered rings. These configurational units may be linked through edges to form chains and the chains to form a three-dimensional framework which describes an intersecting channel system with openings

Fig. 1 Scanning-electron micrograph of a ZSM-11 crystal. Scale bar, 1 μm .

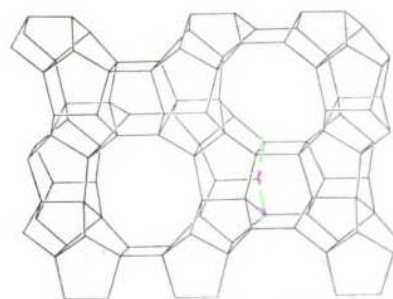
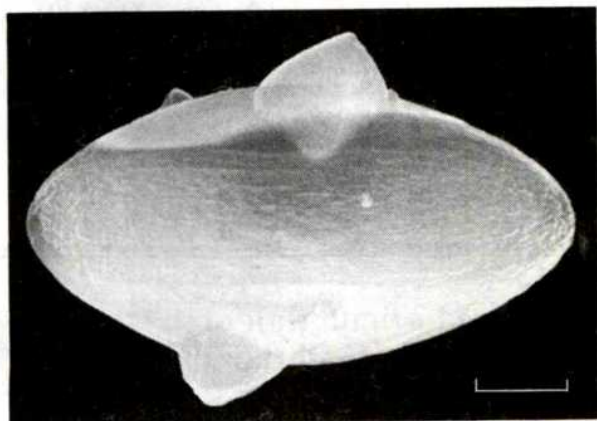


Fig. 2 Skeletal diagram of the A and B faces of the ZSM-11 unit cell. The c -axis is vertical. Oxygen atoms are not shown. The nearly circular 10-membered ring apertures shown are the entrances to straight channels which run parallel with the unit cell a and b axes.

intermediate between those in zeolite Linde type A and faujasite. We report here the synthesis and structure of another member of this family, ZSM-11.

ZSM-11 was hydrothermally synthesised in a system consisting of a quaternary compound, sodium oxide, an oxide of aluminum and oxide of silicon and water⁸. The single crystals of ZSM-11 have the characteristic morphology seen in Fig. 1. In the synthesised form, ZSM-11 has a typical empirical composition $[xR_4X + (1-x)M_{2/n}O]:W_2O_3:>10YO_2:ZH_2O$, where M is an alkali metal cation, W is aluminum or gallium, Y is silicon or germanium, R_4X is a cation of a quaternary compound of an element of group 5A, and $0 < x \leq 1$. Typical quaternary compounds used in the synthesis are tetrabutyl phosphonium chloride, benzyltriphenyl phosphonium chloride and tetrabutyl ammonium iodide.

The unit cell contents of the sodium form are $Na_nAl_nSi_{96-n}O_{192} \cdot \sim 16H_2O$, where n is < 16 . The resulting framework density is $17.6Si + Al \text{ k}\text{\AA}^{-3}$, compared with 17.9 for ZSM-5.

ZSM-11 crystallises in the tetragonal system with lattice constants $a = b = 20.12$ and $c = 13.44 \text{ \AA}$ which corresponds to

Fig. 3. X-ray diffraction patterns: a , computed from parameters in Table 1; b , of calcined ZSM-11.

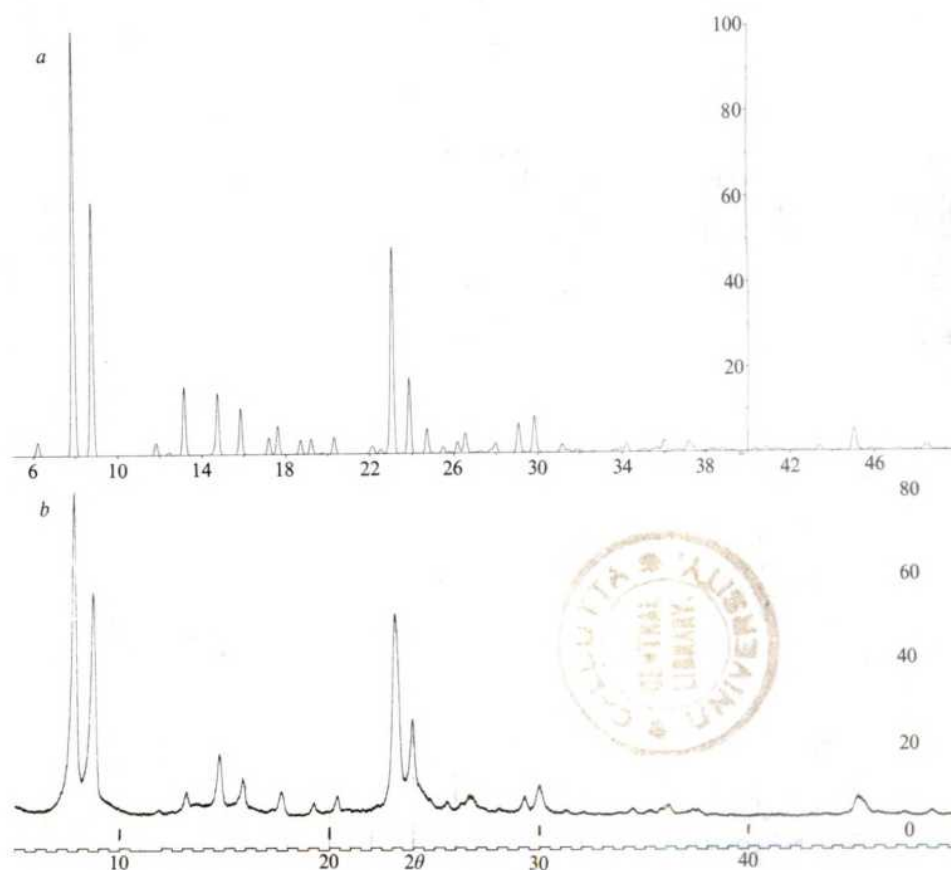


Table 1 Positional parameters for ZSM-11 framework

Atom	Position	x	y	z
T1	8g	0.0774	0.0774	0.0
T2	16j	0.1263	0.1930	0.1263
T3	16j	0.0784	0.2245	0.3477
T4	16j	0.2833	0.1969	0.1321
T5	16j	0.3118	0.0742	0.0089
T6	8g	0.1796	0.1796	0.5000
T7	16j	0.0770	0.3811	0.3610
O1	8i	0.0924	0.0	0.0208
O2	16j	0.0989	0.1207	0.0954
O3	16j	0.1115	0.2063	0.2422
O4	16j	0.2051	0.1965	0.1065
O5	16j	0.3157	0.1273	0.0981
O6	8i	0.3068	0.0	0.0533
O7	8h	0.2933	0.2067	0.2500
O8	16j	0.1126	0.1810	0.4342
O9	16j	0.2431	0.1814	0.4271
O10	16j	0.0895	0.3023	0.3707
O11	8i	0.0	0.2087	0.3441
O12	8i	0.0	0.3970	0.3836
O13	16j	0.0892	0.2480	0.0596
O14	16j	0.1230	0.4198	0.4400
O15	8h	0.0951	0.4049	0.2500

average values derived from a least-squares refinement of X-ray powder diffraction data. The systematic extinctions $h + k + l = 2n + 1$ for hkl reflections and the merging of doublets into singlets provided two major clues that ZSM-11 was a species distinct from ZSM-5 and a body-centred tetragonal variation of orthorhombic ZSM-5 with space group $D_{2d}^{14} - I4m2$. Accordingly, a framework model was assembled using the chains in ZSM-5 (ref. 2), which satisfied these symmetry requirements. This was accomplished by connecting the chains together as mirror images of one another in both x and y directions (Fig. 2). In contrast, in ZSM-5 only one set of chains, in the bc plane, are mirror images of one another. The atomic positions of the silicon and oxygen atoms in this model were refined by least-squares techniques using the computer program DLS.⁹ This refinement adjusted the raw positional coordinates until the Si-O, O-O, and Si-Si interatomic distances fit the prescribed distances 1.61, 2.63 and 3.20 Å, respectively. The final positional parameters for this idealised model are given in Table 1. Comparison of the simulated powder pattern of the framework model with the X-ray powder diffraction pattern of the hydrogen form of ZSM-11 (Fig. 3) indicates that the framework model is that of ZSM-11.

In ZSM-5, linkage of chains occurs through 4-, 5-, and 6-membered rings, whereas in ZSM-11 this linkage occurs only through 4- and 6-membered rings. Nevertheless, the frequent

occurrence of 5-membered rings throughout the structure is believed to account for the high thermal stability of ZSM-11.

As in ZSM-5, the ZSM-11 framework contains two intersecting channel systems with 10-membered ring openings, but unlike those in ZSM-5 they are both straight and have the same elliptical opening with free diameter intermediate to those in Linde type A and faujasite (Fig. 4).

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A preferred orientation of intrusion of Precambrian dykes

AN analysis of the original orientation of Precambrian dykes has produced a nonrandom pattern that implies the existence of a Precambrian stress field of global extent. In general, dyke intrusion tends to occur in a north-south orientation, and is concentrated in low latitudes. This distribution has many similarities to the present distribution of sheeted diabase complexes of present day oceanic ridge crests, and may reflect the persistence of the same stress field throughout geological time. Other evidence in favour of the existence of this stress field can be found in tectonic motions or geological features that exhibit a symmetrical distribution relative to the Earth's rotational axis. For example, Moore¹ showed that the preferred orientation of spreading axes is directed north-south with a secondary maximum orientated east-west. Active transform faults exhibit a unimodal east-west distribution. A former study of recent continental rift valleys has revealed a preferred north-south orientation of fracturing. Cainozoic grabens, most of which have not been significantly rotated by plate movements following their formation, exhibit an especially strong peak within 10° of north². Here we describe our analysis of preferred fracture orientations using the trends and directions of Precambrian dykes. A central point of this study is the assumption that regional dyke patterns record fractures which result from regional stress fields operative at the time of dyke intrusion. Escher *et al.*³ have confirmed the above assumption using geological evidence from the Amitsoq dykes of Greenland.

On observing that dyke strike and magnetic declination were often closely related, Strangway⁴, proposed that the planar shape of dykes affected their acquired remanent magnetisation direction. Thus it was suggested that dykes do not record the true field direction at the time of their intrusion. This theory can no longer be sustained for two reasons. First, positive contact tests show that the magnetisation of country rocks baked by the dyke intrusion retain the same remanence direction as the dyke. Second, palaeomagnetic poles derived from dyke rocks agree

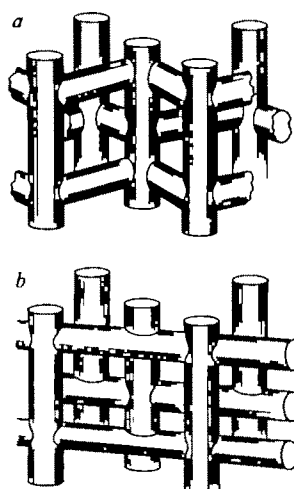


Fig. 4 Comparison of the channel structure in a, ZSM-5 and b, ZSM-11

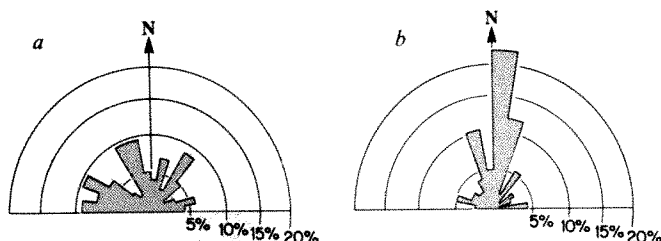


Fig. 1 Rose diagrams to show: *a*, the present distribution of the strike of Precambrian dykes; and *b*, the distribution of dyke strikes after correction to their original orientation.

well with poles derived from adjacent contemporaneous lava flows and sills.

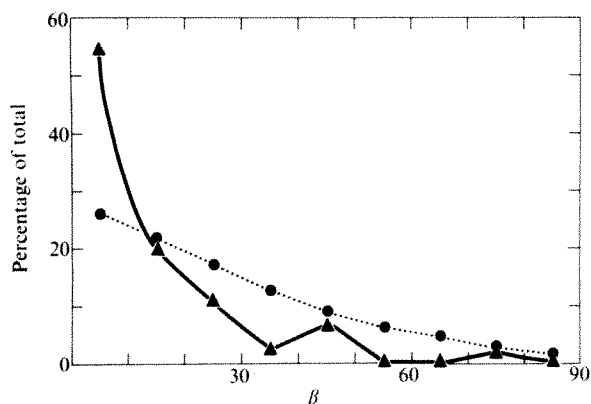
If Strangway's initial observation were still valid, then an alternative explanation is required for the direct relationship between original dyke strike and its remanence direction. The obvious inference is that dykes are preferentially intruded in a north-south orientation and their apparent present-day random distribution is a result of complex plate rotations subsequent to each phase of dyke intrusion. Using the palaeomagnetic remanence declination (D) and the present day dyke strike (S_p), the original dyke orientation (S_0) should simply be $S_0 = D - S_p$. World Precambrian dykes, from Archean to Hadrynian, which have been studied palaeomagnetically⁵ were combined on two rose diagrams. The data for the diagrams include dykes from both the Northern and Southern Hemispheres. As strikes are bimodal, all points are plotted in the range 270–090°. Each spot represents a swarm of dyke intrusions (such as, the Abitibi swarm, and the Sudbury dykes). Where a dyke swarm is composed of a conjugate set this is represented by two points. Where more than one study has been reported from a particular dyke suite, this is plotted as only one point.

Figure 1*a* shows the dykes in their present orientation (S_p), and Fig. 1*b* shows the distribution of the corrected dyke orientations (S_0). A simple comparison shows that a random distribution of strikes in Fig. 1*a* is brought into a clear north-south orientation in Fig. 1*b*.

Evans⁶ has noted that with increasingly higher latitudes there is an increasing statistical chance of a coincidence between original dyke strike (S_0) and remanence declination (D). Thus a random sample of dykes from subsequently higher latitudes should show a progressively increased preference for a north-south orientation. Could this explain our observed distribution? It is highly unlikely. Applying the simple relationship between magnetic inclination and palaeolatitude shows that 85% of the dykes analysed in this study have palaeolatitudes below 50°, and 40% are within 20° of the Equator.

Fig. 2 Frequency distribution of the acute angle (β) between the dyke plane and the magnetic remanence vector. The theoretical curve after Evans⁶ is for a set of vertically intruded dykes with random strike. The over-abundance of low β observations suggests the preferred north-south orientation is of geological significance.

●, Theoretical; ▲, observed.



The observed frequency distribution (Fig. 2) of the acute angle (β) (ref. 6) bears no similarity to the predicted distribution given by Evans⁶ for random orientation of dyke intrusion. As shown in Fig. 2 the expected frequency of $\beta < 10^\circ$ on a random dyke orientation model is approximately half the observed distribution. Hence it would seem that the preferred north-south mode of intrusion genuinely reflects a feature of the Precambrian global stress field. The cause of this north-south preference of dyke intrusion remains obscure; however, we note that the distribution is very similar to that now observed with sheeted diabase complexes of the ocean ridges.

Finally, as shown in Fig. 1, much of the present apparent random dyke orientation seems due to post-intrusional rotation of continental blocks. Then, where dykes from adjacent cratonic blocks can be shown to be recording contemporaneous remanences, it would be simple to determine the amount of relative rotation between the blocks since remanence acquisition (dyke intrusion). Applying this method to several dyke pairs offers the possibility of defining the sense of motions of major fault systems through geological time.

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Oxygen isotope palaeotemperatures from the Tertiary period in the North Sea area

THE understanding of global temperature conditions during the Tertiary period has greatly increased in the past decade owing to the successful oxygen isotope investigations of the numerous deep sea cores now available. Little attention has been paid, however, to applying the method to material from shelf sea areas, where the temperature-induced ^{18}O -record in carbonate shells are generally believed to be superimposed by ^{18}O -variations in water composition and by postdepositional alterations. This is unfortunate, as oxygen isotope data from coastal areas provide the best way of comparing traditional palaeoclimatological methods with the oxygen isotope procedure. The present study, which deals with the oxygen isotope composition of shell material from the Tertiary North Sea area, was initiated to overcome this lack of data. Here the oxygen isotope results are interpreted as palaeotemperatures and the first isotopic palaeotemperature curve for the Tertiary period in NW Europe is given.

The only reliable method of absolute palaeotemperature determinations is based on the temperature dependence of oxygen isotope exchange processes between water and shell carbonate¹. Most oxygen isotope palaeotemperature studies published recently have used foraminifera collected from the deep sea cores^{2,3}. Using foraminifera for palaeotemperature studies is difficult, however, in shelf sea areas. First the number of planktonic foraminifera is greatly reduced owing to the more restricted character of such seas⁴. Moreover, exchange processes affecting the original isotopic composition of the thin-walled foraminiferal tests are far more probable in diagenetic environments open to meteoric water than in deep sea samples.

The present study has been restricted to shell carbonate from molluscs. They are abundant as fossils in the area, and their size and often primary aragonitic mineralogy allow post-depositional alterations to be easily identified⁵. Moreover, they are known to secrete shell carbonate in isotopic equilibrium with the surrounding water⁶.

Oxygen isotope data have been obtained from samples of individual shells or combined shell assemblages collected in Tertiary strata in southern England, Holland, Germany, Denmark and southern Sweden. The material was dated by reference to the stratigraphic scheme summarised by Berggren⁷.

The isotopic results presented in Fig. 1 disclose a well defined pattern of variations in the ¹⁸O-composition of shell carbonate during the Tertiary period. This pattern, however, may reflect variations in oxygen isotope composition of the Tertiary North Sea water as well as in temperatures⁸. According to Shackleton and Kennett³, the accumulation of glacial ice on Antarctica has changed the average ¹⁸O-composition of ocean water by as much as 1‰ since middle Miocene time. Moreover, local deviations in oxygen isotope composition of seawater due to evaporation and fresh water mixing processes have to be taken into account. These processes vary in parallel to salinity^{9,10} and, consequently, palaeotemperature calculations have to be preceded by estimates of the salinity of the water in which the organisms grew their shells. This imposes a very severe limitation on the oxygen isotope palaeotemperature method, as no reliable procedure of palaeosalinity determination has yet been developed.

The present study has been restricted to material from faunal assemblages, the composition of which indicates normal marine environments at a certain distance from the coast. Minor fluctuations in salinity, however, are not detectable in this way. The average salinity in the Tertiary North Sea is assumed here to have varied within limits of 33–37‰, corresponding to variations in oxygen isotope composition of ~1‰ (ref. 10). The corresponding maximal error in isotopic temperatures is ~4 °C.

The palaeotemperature curve shown on Fig. 2 reflects these limitations. It is important to emphasise the fact that most of the ¹⁸O-data have been obtained from benthonic organisms and therefore reflect bottom temperatures rather than surface conditions. However, when compared to modern conditions the investigated assemblages in most cases represent soft-bottom communities of moderate depth (30–150 m) (ref. 11). At such a depth no well defined thermocline is thought to have developed and hence—as seen in the present day North Sea—bottom temperatures are supposed to be good approximations to mean annual surface temperatures.

The isotopic palaeotemperature curve (Fig. 2) illustrates water temperatures in the southern part of the Tertiary North Sea. Temperature minima are seen in Palaeocene time (NP 4–6), middle Oligocene time (NP 22–23) and late Miocene time (NN 10), while warmer conditions prevailed in Pliocene, early and middle Miocene and specifically in Eocene time. Minor fluctuations on the curve probably reflect variations in the isotopic composition of the North Sea water.

Several studies dealing with the changing character of fossil floral and faunal communities have revealed that long-term climatic fluctuations occurred in NW Europe during the Tertiary period^{12–14}. According to these studies subtropical climates in Palaeocene time and warm subtropical to tropical conditions in Eocene time were followed by a gradual decline in temperatures, leading to cold temperate conditions at the end of the Pliocene period. Compared to these data, the temperature variations seen in Fig. 2 indicate a more drastic pattern than hitherto described.

The present curve shows discrepancies between palaeotemperature and palaeoclimatological data specifically in Oligocene time. Water temperatures are seen to have declined rapidly in the North Sea about 36–38 Myr ago. This decline may reflect the onset of cold-water circulation in the Norwegian–Greenlandic Sea initiated by the opening of the strait between Greenland and Svalbard at anomaly 13 time (37–38 Myr ago)¹⁵. However,

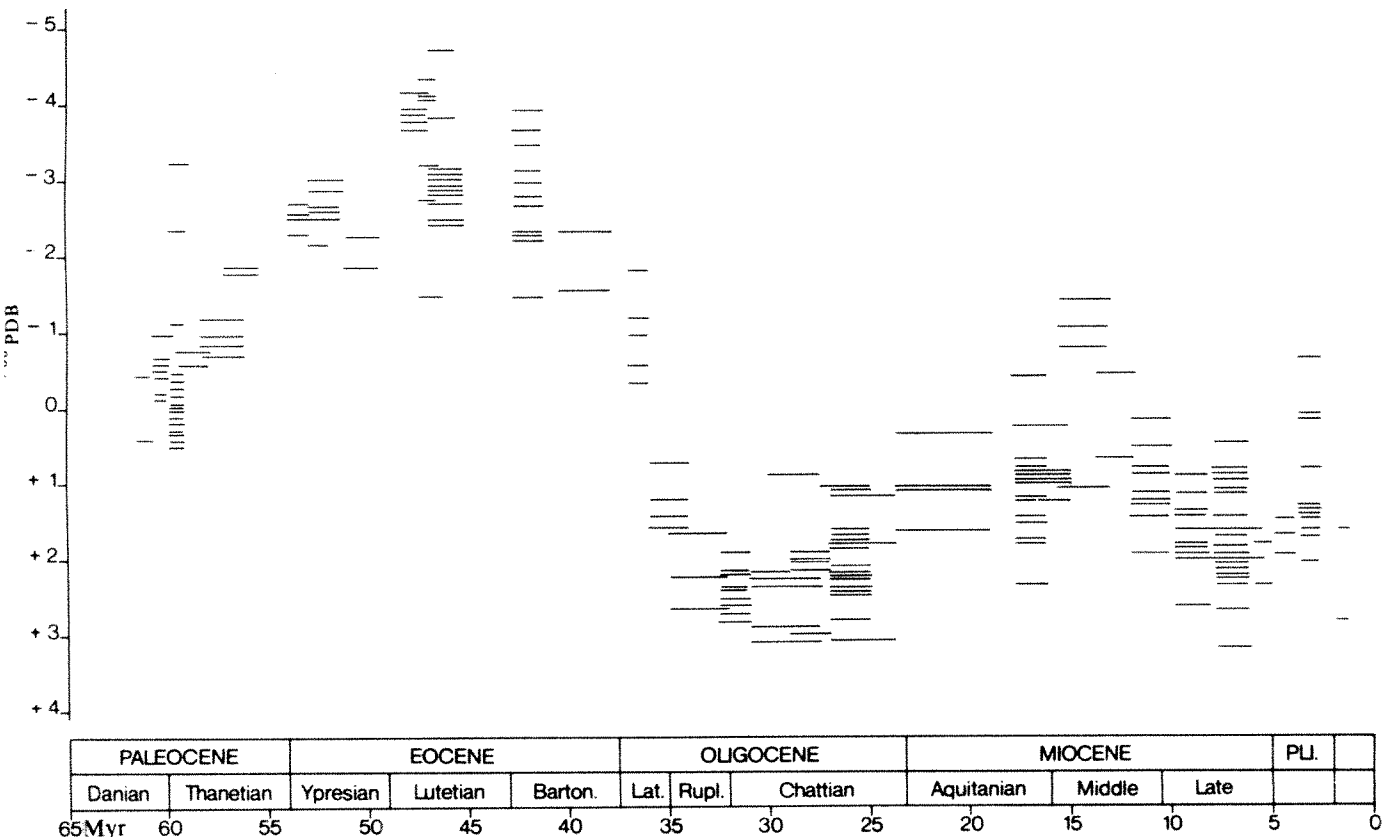


Fig. 1 The oxygen isotope composition of calcium carbonate from molluscan shells collected in Tertiary outcrops and borings in NW Europe. The horizontal bars illustrate the uncertainty in dating the analysed material. Oxygen isotope determinations have been carried out by the usual analytical procedures, material has been roasted *in vacuo* and treated with 100% phosphoric acid at 25 °C. Analytical precision better than ±0.1‰.

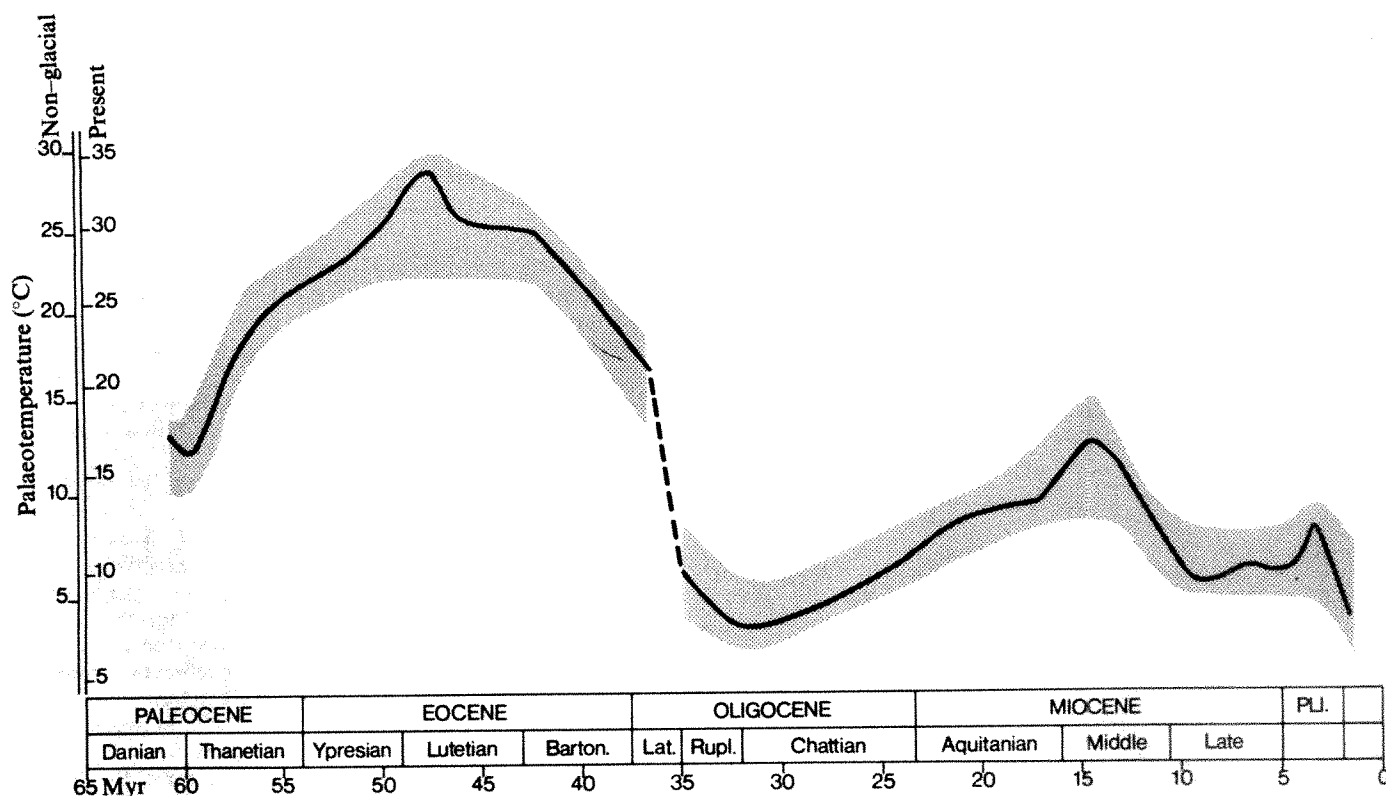


Fig. 2 Isotopic temperatures calculated from average oxygen isotope values for each locality investigated. Shaded area represents limits of uncertainty due to local variations in the oxygen isotope composition of the Tertiary North Sea water. True Tertiary water temperatures are believed to be within the shaded area. The difference between the non-glacial and the present temperature scales is caused by the shift in average oxygen isotope composition of ocean water, owing to the accumulation of glacial ice on Antarctica. According to Shackleton and Kennett³ the Antarctic icecap reached present day magnitude early in the middle Miocene.

convincing evidence from other parts of the world indicates a drastic global cooling event at the beginning of the Oligocene period, probably triggered by the development of the cryosphere¹⁶⁻¹⁸. If the unresolved problem of the stratigraphic position of the Latdorfian substage (Upper Eocene or Lower Oligocene) in NW Europe were taken into account, the isotopic shift observed between shell material from Latdorfian and Rupelian deposits may prove to be contemporaneous with the global cooling event. Thus, the low water temperatures in the Oligocene North Sea may be interpreted as being in phase with global climatic changes.

Further investigations should concentrate on the comparison between palaeoclimatological data obtained by traditional methods and the oxygen isotope curve, specifically for the Oligocene period where the extensive climatic modifications are expected to have influenced faunal and floral compositions much more than previously acknowledged. Moreover, the precise identification in NW Europe of the Oligocene global cooling event could prove to be a valuable chronostratigraphic tool in the solution of the Latdorfian Problem.

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Test size variation in *Globigerina bulloides* in response to Quaternary palaeoceanographic changes

SEVERAL different methods have been used to delineate Quaternary palaeoclimatic trends in deep-sea sediments using phenotypic variation in planktonic foraminifera including changes in coiling direction¹, pore concentration², and test surface ultrastructure³. Several workers have observed that test sizes in planktonic foraminifera may differ between different water masses⁴⁻¹⁰ but little attempt has been made to use size variations as palaeoclimatic indices within the Quaternary. The exception is based on size variations in the spherical test of *Orbulina universa* in late Quaternary deep-sea cores from the southern Indian Ocean¹¹. Oscillations in mean diameter of this species closely match late Quaternary palaeoclimatic changes: larger mean diameters occurring during warm episodes. We

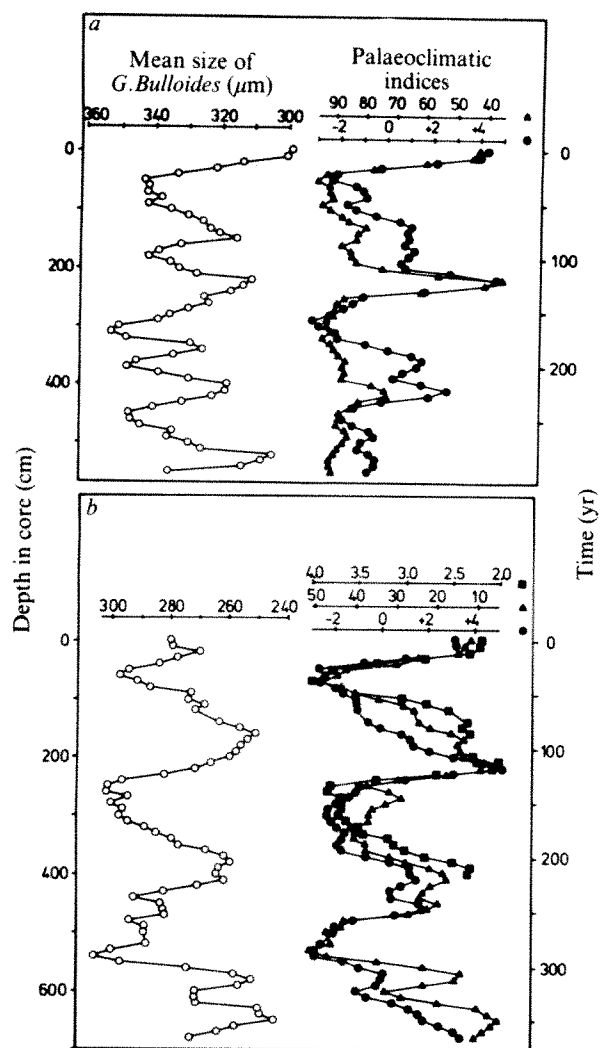


Fig. 1 Variation in mean sizes of *G. bulloides* in relation to three palaeoclimatic indices in cores Eltanin 49-19(a) and 48-22(b) from the southern Indian Ocean. The curves are plotted as three-point moving averages. 'Warm' is to the right and 'cool' to the left. Oxygen isotopes and other data for E48-22 are from Williams¹⁴. Chronology plotted on right is based on extrapolation of ages (assuming constant sedimentation rates) from Termination II (128,000 years ago¹⁵) which is at 235 cm in E49-19 and 236 cm in E48-22. ●, Palaeoclimatic principal component; ▲, % sinistral *N. Pachyderma*; ■, oxygen isotope ratios ($\delta^{18}\text{O}$)

report here that changes in mean sizes of *Globigerina bulloides* can also be used as a palaeoclimatic index in the late Quaternary.

We have examined mean size oscillations of *G. bulloides* in three late Quaternary cores from sub-Antarctic waters of the southern Indian Ocean¹² (Table 1). The results from two of the cores are presented here. The third core examined (E45-74) shows conspicuous evidence of calcium carbonate dissolution, and the data revealed no meaningful relationships between size parameters and climatic history. Previously⁸ we have shown that in surface sediments of the southern Indian Ocean, *G. bulloides* increases in mean size with decreasing surface-water temperature, and we wished to test if this trend could be usefully applied to down-core palaeoclimatic studies. The mean size oscillations were compared in both cores with palaeoclimatic curves established from principal component analyses of abundance data of planktonic foraminifera¹³ and frequency changes in coiling proportions of *Neogloboquadrina pachyderma*, and in E48-22 also with a curve based on $^{18}\text{O}/^{16}\text{O}$ ratios.

In core E49-19 from the central sub-Antarctic, the mean size oscillations of *G. bulloides* are very similar in period and amplitude to the palaeoclimatic curves (Fig. 1) indicating a strong

Table 1 Data for Eltanin cores used in the study

Core	Latitude (S)	Longitude (E)	Water depth (m)	Core length (cm)	Age of sequence (kyr)
E48-22	39°53.7'	85°24.6'	3,380	680	370
E49-19	43°53.2'	90°06.0'	3,036	550	300

palaeoclimatic control of the mean size variation. A cross-correlation coefficient of 0.63 ($P < 0.1\%$) indicates that the curves are statistically significantly correlated. Mean sizes are larger during cool episodes and smaller during warm episodes; this pattern therefore agrees with the distribution in surface sediments⁸. Thus, latitudinal shifts in water masses during the late Quaternary created latitudinal displacements of isopleths of mean sizes of *G. bulloides*.

In core E48-22 from the Subtropical Convergence, there are also strong correlations among the mean size curve of *G. bulloides* and the palaeoclimatic indices (Fig. 1). However, in contrast to E49-19, the mean size curve is not exactly in phase with, but is slightly off-set from the other curves. Cross-correlation analyses indicate that it is off-set from the variations in coiling ratios of *N. pachyderma* and oxygen isotopic ratios by 10 cm (to ~5,500 yr) and from the palaeoclimatic principal component by 40 cm (to ~22,000 yr). These differences do not, however, impair the almost perfect correlation between mean sizes and climatic history.

Palaeotemperatures have been estimated for E49-19 and E48-22 using the following palaeotemperature equation established from the mean size variation in *G. bulloides* in surface sediments⁸:

$$\text{Temperature (annual-average surface-water in } ^\circ\text{C)} = -0.1184 (\text{mean width of } G. \text{ bulloides in } \mu\text{m}) + 45.06.$$

The calculations show that surface-water temperatures ranged between about $3^\circ\text{C} \pm 1^\circ\text{C}$ and $10^\circ\text{C} \pm 1^\circ\text{C}$ at the site of E49-19 (central subantarctic) and between about $9^\circ\text{C} \pm 1^\circ\text{C}$ and $16^\circ\text{C} \pm 1^\circ\text{C}$ at the site of E48-22 (Subtropical Convergence). Our estimates for E48-22 agree closely with the temperature range of 6°C to 15°C calculated previously for this core using different parameters¹⁴.

We have therefore shown that mean size of *G. bulloides* in the late Quaternary of the subantarctic is strongly related to palaeoclimatic trends, and hence this parameter represents a potentially valuable new tool for palaeoclimatic analysis in this area.

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Genetic differentiation during speciation

SPECIATION theory is still largely descriptive. How many and what kind of genes are implicated in speciation is a central unresolved problem of evolutionary biology^{1,2}. Does speciation require major genomic changes³⁻⁵ or may minor ones suffice^{1,6-9}? Similarly, does speciation depend on structural or on regulatory genes¹⁰? We have investigated these questions with reference to the actively speciating fossorial mole rats of the *Spalax ehrenbergi* complex in Israel, which comprises four morphologically indistinguishable chromosome forms ($2n = 52, 54, 58, 60$)¹¹ adapted in that order to increasing aridity¹². Narrow hybrid zones between karyotypes¹³ and mate selection¹⁴ (through olfaction¹⁵, vocalisation¹⁶ and aggression¹⁷) suggest that the recently¹⁸ formed species represent progressive stages of final speciation¹³. Genic diversity proved low, and genic similarity between karyotypes very high, in the previous test based on 17 gene loci of tissue proteins⁸. The test of eight additional loci of blood proteins, which is reported here, reinforces earlier conclusions and sheds light on the allozyme-environment association and on the amount of genetic differentiation during speciation.

In the previous study⁸, 383 adult mole rats comprising all four karyotypes were tested for 17 gene loci. In the study reported here blood samples of 499 specimens, collected during 1969-1971 from 21 populations of the four karyotypes, were tested for eight gene loci, six in the serum (haptoglobin, *Hp*; transferrin, *Tf*; albumin, *Alb*; slow α_2 globulin (*Gl*); esterases, two scorable loci, *Est-1* and *Est-4*) and two in the haemolysate (haemoglobin, *Hb*, and a non-haemoglobin erythrocyte, *Prot-1*). Routine vertical starch gel electrophoresis was conducted on the separated blood samples. Details of collecting sites and electrophoretic methods and results are available from us. Of the eight loci examined, six (*Hp*, *Gl*, *Alb*, *Est-1*, *Hb* and *Prot-1*) were monomorphic and fixed for the same allele in all 21 populations tested. Only two loci, *Est-4* and *Tf* were polymorphic, with three segregating alleles in each. Transferrin is the only locus, both of the blood and tissue proteins, which displays almost complete alternative fixation: $2n = 54$ is nearly fixed for Tf^a , whereas $2n = 52, 58$ and 60 are either fixed or nearly so for Tf^b ; the rare allele, Tf^c , appears only in $2n = 60$. Mean genetic indices for the *S. ehrenbergi* complex and for $2n = 52, 54, 58$ and 60 , respectively, are: *A* (mean number of alleles per locus) = mean 1.25 (1.16, 1.28, 1.20, 1.36); *P* (mean proportion of polymorphic loci per karyotype; criterion for $P 1\%$) = mean 0.20 (0.12, 0.24, 0.16, 0.28), and *H* (mean proportion of loci heterozygous per individual) = mean 0.039 (0.035, 0.016, 0.037, 0.069). Genetic identity, I^{19} , between the four karyotypes, is remarkably high (mean 0.966, range, 0.931-0.988).

The results support the hypothesis that habitat specialists display significantly lower genic variation than habitat generalists²⁰. Mole rats, like most fossorial mammals⁹, are habitat specialists living apparently in a relatively predictable environment which presumably selects for homozygosity (homoselection), though exceptions exist²¹. The evidence negates genetic drift due to small population effects, inbreeding in relatively semi-isolated populations, or the time hypothesis²² as explanatory models. First, the genetic pattern is characterised by general uniformity of alleles at 24 out of 25 loci in 21 populations across the range of the four abundant karyotypes. Second, *H* is apparently not correlated with time. Evolutionary divergence time for the *S. ehrenbergi* complex, deduced from electrophoretic data²³, is within the last $250,000 \pm 20,000$ yr, in accord with the hybrid zone¹³ and fossil²⁴ evidences. Yet $2n = 60$, presumably the last derivative of speciation 75,000 yr ago, has the highest *H*, not the lowest as expected by the time divergence hypothesis²². Heterozygosity seems to correlate with spatial environmental heterogeneity. The largest range, that of $2n = 60$ (see map in ref. 11), is also the most variable

spatiotemporally in terms of ecological heterogeneity and uncertainty, extending in both Mediterranean and arid environments.

Concerning speciation theory, the *Spalax ehrenbergi* complex represents a remarkable case of speciation with relatively few genomic changes. The nature and extent of hybridisation in *S. ehrenbergi*¹³ reinforces the hypothesis that the chromosome forms are young, closely related species at final stages of speciation. A growing body of evidence from newly formed unrelated plant and animal species²⁵ suggests that the essence of speciation, that is the development of pre- and/or postmating reproductive isolation *per se* may occur with little genomic change, either genic or chromosomal. In many cases the apparent genetic distance between species is the result of post-speciation evolutionary divergence. In the fossorial rodent complexes of *Thomomys talpoides*⁶ and *S. ehrenbergi*, where all stages of evolutionary divergence from large to zero gene flow between diverging populations are known, further development of ethological isolating mechanisms based on the few chromosomal rearrangements which initiated the reproductive isolation have been accomplished without many allelic changes. This pattern is also known in other species²⁵, including some parasites whose speciation involves few changes in either structural or regulatory genes, and also rarely involves chromosome rearrangements². The hypothesis that regulatory rather than structural genes are implicated in speciation¹⁰ is yet to be tested. Until tests of regulatory genes become available it seems plausible to assume that speciation may occur with relatively little genetic change.

Species vary in kind in accord with different strategies of evolutionary adaptations and speciation patterns. Reproductive isolation may either follow or precede the genic or chromosomal divergence of gene pools²⁶, and so speciation may be associated with either major or minor alterations in structural and/or regulatory genes, as well as with chromosomal evolution. The extent of genetic differentiation depends on various correlated and interacting factors, including the taxon, mode and rate of speciation; population and migration size and structure; breeding and mating system; reproductive strategy, chromosomal rearrangements and polyploidy, and the ecological selection structures. To become both predictive and falsifiable, speciation theory must be based on critical multifactorial tests incorporating the genetic system, ecological regime and historical patterns in actively speciating populations.

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Limit of single vision in stereopsis depends on contour sharpness

OUR two eyes see slightly different images of the world, and this enables us to estimate the position of objects in depth. When we look at a fixed point in space we can correctly judge the distance of an object that is in front of, or behind this point by the amount of disparity of its retinal images. Our brain combines these disparate images in such a way that we can see the object as single within a certain limit of disparity. This limit is called Panum's fusional area; for fine-line targets the disparity limits are found to be ± 7 min arc for central (foveal) vision, and larger for peripheral vision (for review see ref. 1). However, Fender and Julesz² demonstrated that fusion may be extended beyond Panum's area when random-dot stereograms are used. Experiments reported here demonstrate that Panum's area also increases for stimuli with blurred contours, that is, for contours containing lower spatial frequencies.

Figure 1a shows three gratings of sinusoidal luminance profile; the adjacent gratings are in antiphase, that is, each light bar in one grating corresponds to a dark bar in the other. Each grating is surrounded by a contour and provided with short vertical lines at the top and bottom to facilitate fusion. The first

task of the observer is to fuse the top lines of the top gratings (the bottom left grating and the rest of the page should be covered). Fusion of the top lines is easier when a cardboard partition is put between the gratings, so that each eye views only one grating. It will be seen that after the short lines and the rectangular outline are fused, both the gratings can also be fused (although at first the light bars seem superimposed on dark bars and vice versa). They are seen as a single grating either closer or further away than the short lines (as observed previously by Blakemore and Hague³). The criterion of appropriate fusion is that the top short lines are seen pointing between the light and dark bars of the grating for observers with good binocular vision. (If an observer has one eye strongly dominant, this eye will determine the perceived position of the short line). This fusion is not affected by viewing and fusion of the bottom instead of the top short lines, except that now the short lines are seen in the middle of a light or dark bar of the grating. In both viewing conditions, single vision and stereopsis are easily established over a wide range of viewing distances and this finding was confirmed on 15 observers with normal binocular vision. This indicates that the disparity range of single vision is at least as large as the width of one bar (or a half-cycle of the grating), which at a viewing distance of 10 cm is about 4°.

Now cover the right grating, uncover the left bottom grating and rotate the figure by 90° so that two horizontal gratings (in antiphase) can be viewed by left and right eyes, respectively. It will be seen that now fusion becomes difficult and even if it occurs (most often by suppressing one eye) it does not produce stereopsis.

The next demonstration is concerned with the square-wave gratings (Fig. 1b) in antiphase. Such patterns, with sharply outlined contours, are known to be interpreted in the visual system by detection of their edges⁴⁻⁷, which contain higher spatial harmonics⁸.

First try to fuse the top short lines using the same method as when viewing Fig. 1a. Now, however, a simultaneous fusion of the top short lines and the gratings is very difficult, as the sharp edges of the gratings in antiphase seem to produce rivalry, otherwise the edges are not seen as sharply. In spite of this, all 15 observers saw the square wave grating in depth, either closer or

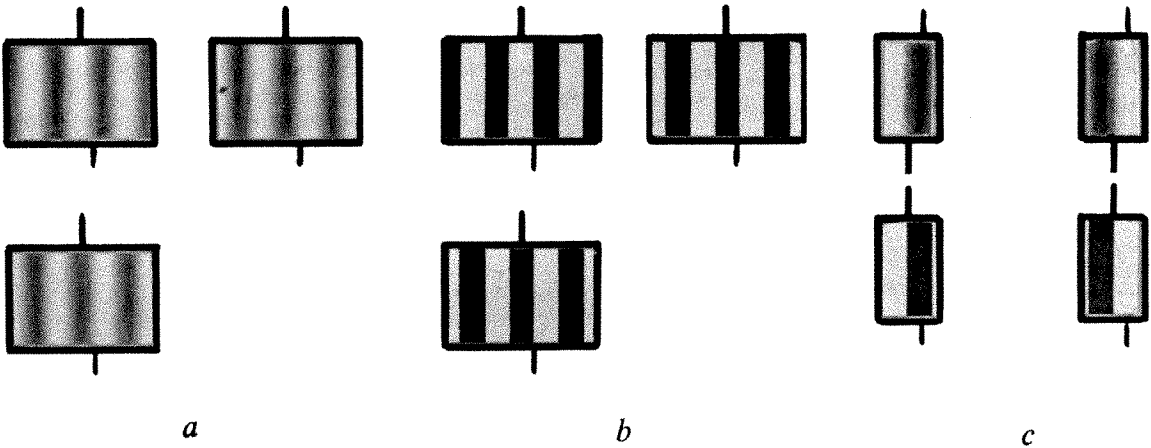


Fig. 1 Demonstration of depth perception with and without single vision of contours. *a*, Sinusoidal gratings in antiphase can easily be fused, after fusing the short line in the top two figures (all other figures should be covered by a white sheet of paper). Observers finding this task difficult should put a piece of cardboard, in the sagittal plane, between their eyes and the two gratings, so that the left and right eyes view only left and right gratings, respectively; start viewing the figure at a distance of 20-30 cm and then gradually reduce the viewing distance. Note that the vertical gratings are fused and perceived at a different depth from that of the short fixation lines over a wide range of distances (down to < 10 cm) and that the gratings do not produce the depth effect if the whole display is rotated through 90°. *b*, Square-wave gratings in antiphase can be fused initially only at a distance over 30 cm, but gradually also at smaller distances. The square-wave grating is then seen in depth relative to the short lines, but the sharp edges of the grating become double or unclear at distances < 15 cm. It is important that the observer sees the gratings in focus with both eyes (otherwise, defocused gratings lose their sharp luminance profile and become similar to the sine gratings). Viewing the gratings horizontally produces only binocular rivalry. *c*, Fusion of single bars of either cosinusoidal or sharp (square) profile is equally easy when looking at the gaps between the short lines in the middle of the figure: both bars are seen as single and in depth. However, the sharp edges of the bars are seen either double or unclear when the observer looks at the bottom short lines at viewing distances < ~10 cm.

farther away than the short lines, and 12 of them reported rivalry at the same time. Only three observers (all of whom had a strongly dominant eye) reported seeing sharp edges without any rivalry, possibly by suppressing the rival contour produced by the nondominant eye. This suggests that the nondominant eye may contribute to stereopsis without producing a rival double image.

Although the periodicity of the patterns in Fig. 1a and b helps in seeing them in depth (as several contours have similar disparity⁹), it is not a necessary condition for this phenomenon. Figure 1c shows two pairs of single bars corresponding to those in Fig. 1a and b. Now, however, for comparison the sharp bars (bottom) and the 'blurred' bars with a sinusoidal profile (top) are presented simultaneously in the same disparity with respect to the short lines. Some observers find it difficult to fuse the short lines between the bars, but after several attempts it is usually possible to perceive the bars as being closer than the short lines (if the left and right eyes look at the left and right lines, respectively). The sharp edges of the square-wave bars, however, are seen as double.

All the phenomena shown in Fig. 1 suggest that objects with sharply outlined contours and patterns with gradual luminance gradients have different ranges of disparities over which they are seen as single, or at least not seen as rival double images. In other words, the range of single vision in stereopsis is inversely related to the spatial frequencies present in the pattern, as though there were many Panum's areas instead of one determined with fine lines.

Neurophysiologists¹⁰⁻¹² have noted that units with small receptive fields, representing central vision, often have a narrower range of disparities than units with large receptive fields representing peripheral vision. However, the phenomena reported here are concerned mainly with central vision (note that the bars in Fig. 1c may be made shorter without affecting the percept). Graham, Robson and Nachmias¹³ have demonstrated that central vision has a range of mechanisms tuned to different sizes (or different spatial frequencies). Also, Maffei and Fiorentini¹⁴ noted that cortical cells subserving each part of the visual field are tuned to a range of sizes (spatial frequencies). Although specific neurophysiological investigations relating the range of disparities to the receptive field size in the same part of central vision have not yet been published, some experiments hint that such a relationship may exist. Simple cells in the cat visual cortex responsive to low spatial frequencies tolerate a greater lateral misalignment of line stimuli presented dichoptically than the cells responsive to higher spatial frequencies (D. J. Tolhurst, J. D. Thompson and C. Blakemore, personal communication).

Other psychophysical experiments also point to different binocular processing of sinusoidal and square-wave gratings in antiphase (J.J.K. and I. Wood, in preparation). The binocular contrast thresholds for the detection of sinusoidal gratings were the same whether the gratings were in-phase (identical) or in antiphase for the two eyes. However the thresholds for binocular viewing of square-wave gratings were higher when the gratings were in antiphase as compared with the thresholds for the in-phase presentation. This finding may be of clinical importance, for as the sinusoidal gratings do not produce rivalry irrespective of their phase they could be used for exercising binocular vision in patients with squint.

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Telephoto lens system of falconiform eyes

VISUAL ACUITY in falconiform birds has been shown to be higher than man. For instance, vultures with eyes similar in size to those of man have a grating detectability of about twice the spatial frequency of man¹. This is consistent with measured image quality in an eagle eye similar in size to the human eye². Recently a falcon (*Falco sparverius*) has been shown to have a grating detectability 2.6 greater than that of man³. Although the image quality of these falconiform eyes is at least twice as good as man, their minimum intercone spacing is only slightly less than in the human retina. Unless the ratio of focal length to the axial eye length of these birds greatly exceeds that in man, falconiform eyes, unlike man, would be unable to resolve their best retinal image quality. We show here that the presence of a spherical depression in the deep fovea of falconiforms may act like the negative lens component in a telephoto lens system or opera glass. The focal length of the birds' dioptries can then in theory exceed the axial length of the eye, thus providing the relatively large images necessary for more complete image reconstruction (high resolving power) in a localised region of the retina.

The anatomical resolving power of an eye is set by the image size and by the receptor grain. The image size is specified by the focal length, f , while the receptor grain is specified by the centre-to-centre spacing of foveal cone, d . It is convenient to define an anatomical resolving power, RP , as the reciprocal of the angular separation between cone centres:

$$RP = f/d \quad (1)$$

In order to determine precisely the intercone distance, d , we made photoreceptor counts by light microscopy on fresh material and by light and electron microscopy on fixed material in the deep foveas of numerous birds ranging from large eagles to small falcons and, for comparison, in human foveas⁴. In all situations shrinkage was carefully monitored and corrected for, and only the most central region of the fovea was examined. In all falconiforms studied $d \approx 2 \mu\text{m}$, independent of their eye size, while $d \approx 3 \mu\text{m}$ in man. In man, focal length $f \approx 17 \text{ mm}$ (ref. 5) while in falconiforms f (believed to be accurately estimated from the curvature of the retina^{6,7}) measured in all our birds was found to be $f \approx 0.65L$, where L is the axial eye length. Using equation (1), the anatomical resolving power of falconiforms with eyes the same size as man ($L \approx 24 \text{ mm}$) is only 1.38 times that of man. If the resolving power of these birds is to equal twice that of man, then f must equal 22.6 mm or 1.45 times that measured from the retinal curvature. We next show that this amount of image magnification is in fact consistent with the optical function of the bottom of the foveal pit.

Falconiforms are well known to have a deep nasal fovea^{8,9}, see for example, Fig. 1, where Fig. 1b shows the deep fovea of the red-backed hawk, *Buteo*, which also has a 'man-sized' eye. We call attention here not to the steep sides of the fovea^{8,10}, but rather to the fact that the bottommost portion of the pit is a well formed hemisphere. Our hypothesis is that this concave portion of the foveal pit (labelled by the arrow in Fig. 1b) functions as a negative lens which, together with the positive power of the cornea and lens, acts like the telephoto optical system of Fig. 2. This system has an effectively long focal length which, in

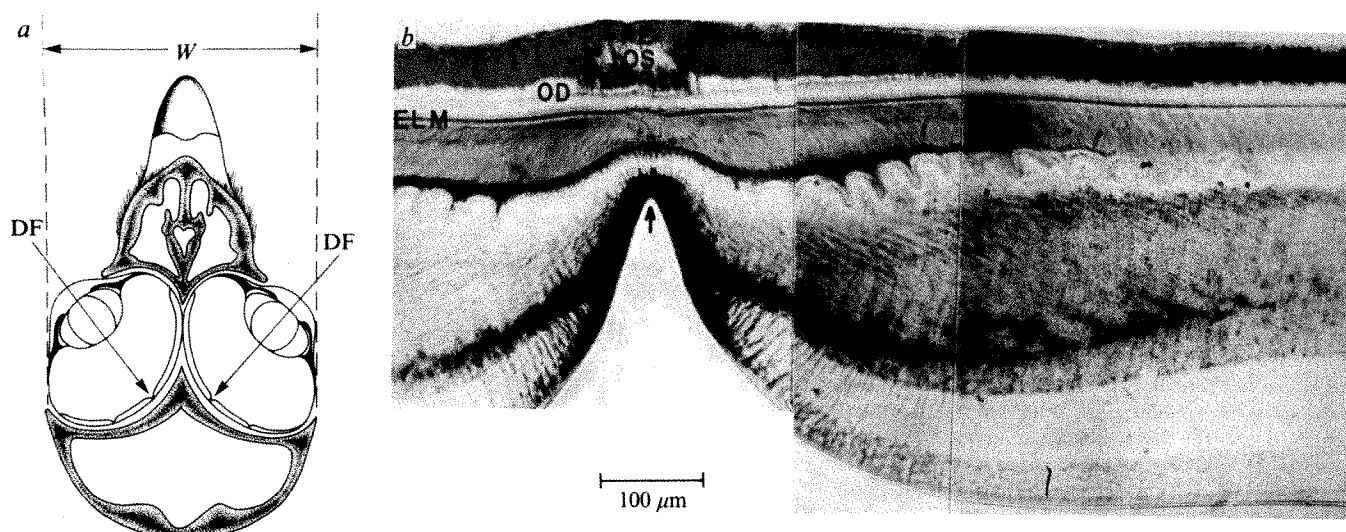


Fig. 1 *a*, Schematic of an avian eye viewed ventrally, illustrating the relative position of the deep or nasal fovea (DF). The figure is adapted from the hawk *Buteo latissimus*¹⁴. *W*, the head width, approximately equals twice the axial eye length *L*. *b*, Unstained 10- μ m thick section of glutaraldehyde-fixed retina of red-backed hawk in region of deep fovea. Section photographed using interference contrast. Increasing darkness indicates higher refractive index. Arrow indicates concave region of foveal pit which we hypothesise may function as a diverging optical element to project magnified image on receptors at centre of fovea. Note that *n* is uniform between internal and external limiting membranes outside of fovea on extreme right of figure. Dark densities sclerad to ELM are the result of pigment and do not necessarily relate to *n*. ELM, external limiting membrane; OD, oil droplets; OS, outer segments; PE, pigment epithelium. Scale bar, 100 μ m.

theory¹¹, can even exceed the physical length of the eye. Walls⁸, ignoring the bottommost portion of the pit, suggested that the steep sides of the foveal pit magnifies the image, but Pumphrey¹⁰ showed that instead they cause distortion.

Thus, from Fig. 2 the effective focal length of the bird eye is given by mf , where m is the magnification¹¹ of the foveal pit, defined in the legend of Fig. 2*b*, and f is the focal length used in equation (1). Therefore, from equation (1), the resolving power of falconiforms is mf/d . The magnification m increases the smaller the radius of curvature R of the concave pit and/or the greater the distance s of the bottom of the pit from the photoreceptors. From our above discussion, m must equal about 1.45 for the man-sized falconiform eye to have twice the resolving power of man.

Using the deep fovea of the red-backed hawk in Fig. 1*b* as an example, with $s = 110 \mu\text{m}$ and $R = 6 \mu\text{m}$, then the refractive index n_r of the deep fovea must equal 1.369 if m is to be 1.45. This result is found from the equation in the legend of Fig. 2*b* assuming the refractive index of the vitreous humor¹² is the same as man and cat⁵, that is, $n_c = 1.336$. There are several convincing reasons why n_r should equal or exceed 1.369. First, the vitreal surface of the foveal pit is formed by Müller cells only. From our electron micrographs, these Müller cells are seen to be exclusively and densely packed with microfilaments. Similar protein, for example, collagen in the cornea, has a refractive index¹² $n_r \approx 1.55$. Second, interference microscopy of the red-backed hawk fovea, (Fig. 1*b*) indicates an n_r similar to that of the cone outer segments¹³ for which $n_r \approx 1.4$. Since the regions of highest n in Fig. 1*b* are shown as the darker areas, the region below the pit has a nonuniform refractive index. Thus, Fig. 2 represents only a crude model of the optics. We emphasise that the high refractive index region surrounding the spherical pit in Fig. 1*b* is absent in extrafoveal regions and in all primate foveas and less apparent in the shallow foveas of falconiforms. Third, measurement of interference fringe displacement in the deep fovea of a glutaraldehyde fixed, Spurr's media embedded specimen (Fig. 1*b*) indicates a minimum value of 1.379 for the refractive index of both the pit and sides of the deep fovea. However, this value is a lower bound because we have not adjusted for the fact that the high n Spurr's medium infiltrates the denser tissue more than the less dense tissue. The measured n value is consistent also with the intensity of the foveal reflex

believed equal to that of myelinated nerve fibres of the optic disk¹⁴. Thus, the very high refractive index below the spherical pit and its radius of curvature are consistent with the pit functioning as a negative element in a telephoto lens system.

The deep fovea may also act as a focus indicator; however, both the nail polish and the ray models used to test this hypothesis¹⁵ were not scaled to give the correct magnification factor as determined by our interference microscopy and anatomical measurements (L. Harkness, personal communication). When appropriately scaled, we have observed the magnification predicted by the telephoto lens model and furthermore, the entire 'retinal' image is in focus in the same plane that magnification occurs.

There are distinct disadvantages associated with the telephoto lens system described above, including a narrow field of view for high resolving power and susceptibility to aberrations caused by the steep sides of the fovea. Why is it then that the avian eye did

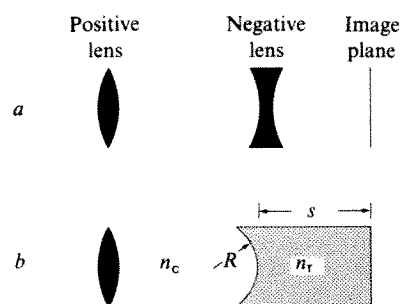


Fig. 2 Schematic of the telephoto lens. *a*, Classical design. *b*, Equivalent system of avian eye where the negative lens is replaced by a spherical (concave) surface. The magnification of the system,

$$m = 1 + \frac{s}{R} \left(\frac{n_r - n_c}{n_c} \right)$$

where n_r and n_c are the refractive indexes of the medium to the right and left of the spherical surface respectively, R the radius of curvature of the surface and s the distance from the apex of the spherical surface to the image plane. The position of the oil droplets is taken as the image plane in Fig. 1*b*.

not simply have a higher cone density, avoiding these difficulties? The answer may be found in the constraints imposed by the wave nature of light. Because of frustrated total internal reflection¹¹, photons within one outer segment have a probability of crossing over into neighbouring outer segments. This optical 'cross talk'¹⁶ increases the more densely packed the cones become, manifesting itself by an increased reduction in contrast sensitivity. If high resolving power is the animals' primary objective, the deep fovea, acting as the magnifier of a telephoto lens, may be a strategy superior to a fine receptor grain.

Although our arguments have been applied mainly to falconiforms with man-sized eyes, they should hold more generally. In all of the falconiforms we have studied, the focal length f of equation (1) is directly proportional to axial eye length L while the minimum inter-cone spacing d and magnification m due to the deep fovea are independent of eye length. Consequently the resolving power, RP , of these birds should be directly proportional to L or equivalently to the animals' head width W of Fig. 1a. Our finding that the birds' pupil diameter, as measured in bright natural conditions, is directly proportional to L is also consistent with the RP being directly proportional to L . This follows assuming, as in man¹⁷, that the birds' best image quality (minimum line spread) is nearly diffraction limited and occurs at the smaller (bright light) pupil diameters.

Finally, recall that grating detectability provides only a limited assessment of bird visual superiority. Alternatively consider a distant point object. The most crucial determinate of detection is then maximum image brightness which also equals the two-dimensional polar area under the modulation transfer function of the optics¹¹. From the modulation transfer function for the optics^{2,17}, we calculate that the man-sized eagle eye can, in theory, detect a distant point object at a distance 3 to 8 times greater than man. This ratio is nearer to 8 when the object is significantly brighter than its surround, and ~ 3 when the object brightness is equal or less than its surround. Such superior visual acuity is in better agreement with the reported observations¹⁸ than the value of twice that of man found from grating detection¹.

Thus man-sized falconiform eyes have an image quality at least twice as good as man², yet their minimum inter-cone spacing is only slightly less than man⁴. Nevertheless, our anatomical findings and refractive index measurements are consistent with the hypothesis that the spherical portion of the deep fovea acts like the negative lens component in a telephoto lens system. The focal length of the dioptrics may then be sufficiently extended for the bird to resolve its superior retinal image in a localised region.

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Cyclophosphamide delays 3-methylcholanthrene sarcoma induction in mice

THERE is increasing evidence to show that suppressor T lymphocytes facilitate the growth of neoplasms that have tumour-specific transplantation antigens¹⁻⁶. Studies on suppressor cell activity with respect to various non-tumour antigens have shown that treatment of mice with cyclophosphamide depresses suppressor cell-mediated mechanisms to a greater extent than the effector arm of the immune response⁷⁻⁹, provided the proper dose and timing for drug administration was used. Based on these observations we speculated that treatment of mice with cyclophosphamide during the latency period of tumour induction might prevent (or at least delay) the appearance of primary tumours. We show here that this hypothesis is correct. In BALB/c mice given 3-methylcholanthrene (MCA) and injected with cyclophosphamide (2 mg per mouse) every 10 days, the appearance of primary sarcomas was delayed. Because the effects were striking, and were obtained with one of the drugs most commonly used in cancer chemotherapy, we decided to report the data. We are, however, aware that explanations for the drug effect other than interference with suppressor cell activity are equally possible, including a direct effect of the drug on tumour cells.

BALB/c mice were bred by brother to sister mating at the Fred Hutchinson Cancer Research Center. Female mice, 6-8 weeks old, were randomised into six experimental groups, each group comprising 50 mice except group 6 which had 45 mice. All mice received an intramuscular injection into each thigh of 0.1 mg MCA dissolved in triolein. The day of MCA injection is referred to as day 1 of the experiment. Two groups (1 and 4) were injected subcutaneously on 4 occasions (days -10, 20, 50, 80) with 10^6 heavily irradiated cells per mouse from tumour 1423. This tumour is an MuLV antigen negative MCA-induced BALB/c sarcoma; these tumour cells were pre-irradiated with 15,000 rad *in vitro*⁶. The tumour cells were injected to determine whether any immunisation to putative common TSTA could be obtained. Two other groups (2 and 5) were similarly injected with cells from normal BALB/c kidneys, while the remaining two groups (3 and 6) received phosphate-buffered saline (PBS) only. Cyclophosphamide was injected intraperitoneally at 10-day intervals in a dose of 2 mg per mouse (corresponding to approximately 100 mg per kg body weight at the onset of the experiment), starting 13 days before MCA and continuing throughout the experiment, in groups 4, 5 and 6. This dose of cyclophosphamide was chosen on the basis of experiments performed in an allograft system, which examined suppressor cell activity controlling the cell-mediated allograft response in BALB/c mice¹⁰. The time schedule was based on the evidence that suppressor T cells have a half life of less than 4 weeks¹¹. Groups 1, 2 and 3 were injected with PBS instead of cyclophosphamide. The previously ear-tagged mice were observed at weekly intervals for tumour development; the treatment

Table 1 Delayed appearance of primary MCA induced sarcomas in mice given cyclophosphamide during the latency period

Group	Cyclophos- phamide* (+ or -)	Immunisation†	No. of mice with tumour at various times after MCA‡						No. of mice dead without tumour d142	Total no. tumours per group d142	% Mice without tumour d142
			d84	d97	d104	d119	d131	d142			
1	-	1423 Tumour	1	28	30	46	48	50	0	73	0
2	-	Normal kidney	4	16	24	44	46	48	1	69	2
3	-	None	6	13	20	41	42	46	4	62	0
1-3	-	1-3 combined	11	57	74	131	136	144	5	204	0.7
4	+	1423 Tumour	1	2	4	18	24	25	3	32	44
5	+	Normal kidney	0	3	3	14	24	31	1	35	36
6	+	None	1	1	2	12	18	26	0	31	41
4-6	+	4-6 Combined	2	6	9	44	66	82	4	98	40
Statistical significance of differences between groups (1-3) and (4-6) <i>P</i> <			0.02	0.001	0.001	0.001	0.001	0.001	NS	0.001	0.001

* 2 mg cyclophosphamide per mouse intraperitoneally 13 d before MCA and at 10 d intervals afterwards.
† 10⁶ cells irradiated with 15,000 rad *in vitro*, were injected subcutaneously 10 d before MCA and at 30 d intervals on three more occasions.
‡ Data pooled from mice with unilateral or bilateral tumours. All groups comprised 50 mice except group 6, in which there were 45 mice. Mice were inoculated with 0.1 mg MCA into each thigh on day 1.

of the mice was unknown to the observer. The appearance and growth of tumours in each thigh was recorded. Statistical significance was calculated by χ^2 analysis.

As shown in Table 1, groups receiving cyclophosphamide developed tumours much more slowly than did untreated groups. At all times of observation except the first (84 days after MCA), the differences were statistically significant at the *P* < 0.001 level; 84 days after MCA, the *P* value was <0.02. At the time of writing, 142 days after injection of MCA, all but one of the mice in the controls had at least one tumour, whereas 40% of the mice in the cyclophosphamide treated group remained tumour free. However, the major effect was probably a delay of tumour appearance rather than a prevention of tumour induction, since the appearance of new tumours in the drug treated group has not levelled off. The cyclophosphamide treated mice have shown no signs of sickness and have increased in weight at the same rate as mice not receiving the drug. The few non-tumour related deaths observed have been no more frequent in the drug treated groups (Table 1).

There was no difference in tumour induction between groups which were immunised with tumour 1423, or injected with normal kidney or PBS. Thus, there was no evidence for any immunisation to antigens shared by the 1423 sarcoma and primary MCA sarcomas. This observation agrees with previously reported failures to immunise against MCA carcinogenesis by giving MCA tumours during the latency period¹², but does not exclude that immunisation with other tumours, either induced by MCA or in another way, may be effective¹³. The failure of 1423 sarcoma cells to immunise against any putative common TSTA was not altered by cyclophosphamide treatment (group 4).

The delayed tumour appearance in groups that received cyclophosphamide is consistent with the hypothesis that cyclophosphamide inhibits tumour growth by interfering with either a suppressor cell mechanism or an immunostimulatory mechanism of the type described by Prehn¹⁴. However, it is equally consistent with a non-immunological interpretation. Cyclophosphamide may, for example, have destroyed neoplastic cells as they developed or it may have interfered with carcinogenesis. That we do not know the mechanisms of the drug effect in this system should not, however, detract from the fact that a very substantial delay in primary tumour induction was obtained, using a protocol which has few apparent side effects. Work in progress in our laboratory, using cyclophosphamide treatment in conjunction with adoptive transfer of lymphoid cell populations containing suppressor cells, should

determine whether the drug has any effect on suppressor cells during chemical carcinogenesis.

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**Neoplastic transformation of human
diploid cells *in vitro*
after chemical carcinogen treatment**

ALTHOUGH many unsuccessful attempts have been made to transform normal human cells to the neoplastic state¹⁻⁴, the putative transformations reported have been primarily with cell lines^{5,6} derived from xeroderma pigmentosum biopsies or osteosarcoma tumour tissues⁷⁻⁹. Recently, cells from a skin biopsy taken from the lip of an adult female were reported to be transformed by 4-nitroquinoline oxide and *N*-methyl-*N*¹-nitronitrosoguanidine¹⁰ (MNNG). We now report that primary or low passage cells *in vitro* from various human foreskin cell populations can be neoplastically transformed *in vitro* by six

different chemical carcinogens. A close correlation between growth in soft agar and tumour formation in nude mice was obtained.

Randomly proliferating human cell populations have an extremely rapid DNA repair system that is exceedingly proficient and error free^{11,12}, as over 90% of the damage caused by the chemical carcinogen is repaired within 4–10 h (ref. 13). When normal diploid human cell populations were exposed to known chemical carcinogens, transitory abnormal cellular, morphological and culture characteristics, such as polygonal morphology, criss-cross orientation of the fibroblast cells and loss of density-dependent inhibition were expressed. We have not yet been able to regularly demonstrate transformation of randomly proliferating cell populations prepared from different skin tissue samples. However, reproducible transformation occurred when scheduled DNA synthesis was altered and proliferation of the normal (unaffected) portion of the cell population was impaired. To obtain transformation the normal cell populations were blocked in G₁/S interphase, then

treatment with PMA, insulin, anthralin or oestradiol. The range for cells in S was 67–95% with a mean value of 90% of the cells going through S phase of the cell cycle for a population of 1.5×10^6 cells in these culture conditions. Carcinogen stock solutions had been prepared by dissolving the chemical in acetone and diluting with 100 ml of CM immediately before use. The concentrations used were made by further dilutions of the stock solutions with CM. After the treatment period, the cell cultures were washed three times with CM and immediately sub-passaged 1:2 into CM containing 8× nonessential amino acids (MEM, Flow) and 2× vitamins. When the cell populations were 75% confluent, the cultures were serially passaged 1:10. This ratio of subpassaging the cultures was continued for 20 population doublings (PDL). The saturation density of the transformed cell populations examined always exceeded the density of the normal untreated cell populations (Table 1).

After 20 PDL, 50,000 cells were seeded into 2 ml of 0.35% agar or 1.0% methylcellulose (6,000 c.p.s.) supplemented with

Table 1 Population density and colony-formation capability in 0.5% agar of control and carcinogen treated cell populations

Chemical carcinogen ($\mu\text{g ml}^{-1}$)	No. of treated populations*	Cell density† ($\times 10^6$ per 75 cm^2)	No. that grow in soft agar‡	Frequency of colony formation in agar
0	—	1.7	—	—
PS (5.0)	7	4.0	7	$1:10^{3.5}$
β -PL (13.0)	4	9.0	4	$1:10^{3.7}$
Af-B ₁ (10.0)	7	4.5	7	$1:10^4$
MNNG (0.1)	8	3.0	6	$1:10^5$
4-NQO (0.002)	10	6.0	7	$1:10^6$
EMS (10.0)	7	8.0	5	$1:10^{3.5}$

The following were added at PDL 1.5: PS, β -PL, aflatoxin B₁ (Af-B₁), MNNG, ethyl methane sulphonate (EMS), 4-nitroquinoline-1-oxide (4-NQO).

* Primaries were derived from 10 different foreskin samples per treatment and cultures of each were used with chemical carcinogens.

† Mean cell population densities (M -cell density $\times 10^6$ per 75 cm^2) were determined 7 d after 1:4 split. These values represent 10 different experiments.

‡ Fifty-thousand cells were seeded into 2 ml of 0.35% agar over a 5 ml 2% base in 25 cm^2 wells. These cultures were incubated at 37 °C in a 4% CO₂-enriched air atmosphere and re-fed on day 7 with 0.5 ml of medium-supplemented soft agar. The base agar medium was supplemented with RPMI 1629 and the soft agar was supplemented with Lo-Cal medium (Biolabs). Colonies, >100 cells per colony, were counted 9 d after seeding.

released and treated with the appropriate carcinogen during the S phase of the cell cycle. After carcinogen treatment proliferation of the unaffected cell population was purposely inhibited. Moreover, if morphologically altered colonies were selectively removed and grown in conditions that restricted proliferation of unaffected cells in mass culture, there was an increase in the growth of the abnormal cell populations.

Randomly proliferating cell populations obtained from 10 foreskin tissues dispersed with collagenase¹⁴ were sub-passaged in complete medium (CM) composed of minimum essential medium–25 mM HEPES (Gibco) at pH 7.2 supplemented with 10% fetal bovine serum (FBS) (Rehatuin), 1 mM sodium pyruvate, 2 mM glutamine, 0.2% sodium bicarbonate, 5.0 $\mu\text{g ml}^{-1}$ gentamycin, in an atmosphere of 4.0% CO₂ enriched air at 37 °C. Preconfluent (70–80%) logarithmically growing cultures were transferred to Dulbecco's modified Eagle's medium (Biolabs) lacking arginine and glutamine, at pH 7.2 supplemented with 10% dialysed FBS to decrease cell multiplication. After 24 h, the amino acid-deficient medium was replaced with CM to which either 0.5 IU of insulin or 1 $\mu\text{g ml}^{-1}$ 17- β -oestradiol, anthralin, or phorbolmyristate-acetate (PMA) had been added. Ten hours later, chemical carcinogen in CM was added with either PMA, anthralin, insulin or oestradiol. This time coincided with the time when the maximum number of cells were in S phase of the cell cycle. Both chemical carcinogen and PMA, anthralin, insulin or oestradiol were removed 12 h later. This time interval coincided with the end of the S phase of the cell cycle (8.3 h). The absolute number of cells in S varied from tissue to tissue and

modified Dulbecco's Lo-Cal growth medium (Biolabs) over a 5 ml 2% base supplemented with RPMI 1629 growth medium–20% FBS. After incubation for 17 d in a 4% CO₂-enriched air environment at 37 °C, colonies formed. Agar-containing cultures were re-fed every 7 d with 0.5 ml of Lo-Cal medium supplemented soft agar. The frequency of colony formation in soft agar ranged from $10^{-3.5}$ to 10^{-6} and for methylcellulose from 10^{-6} to 10^{-7} for the different carcinogen treated cell populations at PDL 20 (Table 1). These colonies were isolated from each semisolid growth medium and seeded into CM containing nonessential amino acids and grown until confluent. When 20,000 cells again were seeded into soft agar, or methylcellulose, the colony frequency approached 100%.

A comparison of the frequency of growth in soft agar, or methylcellulose, indicated that PDL 20, after treatment was discontinued, was a suitable PDL for determining the ability of transformed cell populations to grow in soft agar. Cell populations with a high capacity for growth in soft agar or methylcellulose also grew to relatively higher cell density than normal cultures (Table 1). For example, aflatoxin B₁-treated cell populations at PDL 28 grew in soft agar at $1:10^4$ or methylcellulose, at a frequency of $1:10^{5.4}$ after 17 d. At this PDL the population density in a treated flask was 4.5×10^6 cells per 75 cm^2 after 4 d, while the population density in the control 75 cm^2 flask was 1.7×10^6 cells. At PDL 40 the frequency of growth in soft agar was $1:10^3$ and in methylcellulose it was $1:10^{4.6}$.

After the colonies were removed from the semisolid medium and grown in culture these cells were reseeded into soft agar:

Table 2 Tumour growth in nude mice of same cell lines treated with chemical carcinogens

Cell population	Agar derived cell lines tested	Mice with tumours/mice injected
PS	3	7/11
β -PL	1	3/4
Af-B ₁	3	8/14
MNNG	1	3/5
4-NQO	1	2/4
EMS	1	3/4

At PDL 40–50 the transformed cell populations previously removed from the soft agar were injected into irradiated mice at a cell density of 5×10^6 cells per nude mouse. A 'tumour take' was described as a nodule, 0.5 cm in diameter, that was palpable within 4 weeks after injection. None of the untreated cultures at cell densities of 10^7 cells produced tumours when injected into the mice.

100% of the seeded cell populations formed colonies. A similar response was observed with propane sultone (P.S.), β -propiolactone- or aflatoxin B₁ (Af-B₁)-transformed cell populations, whereas only 25%–70% of the reseeded cell populations, treated with 4-NQO, ethyl methane sulphonate, methyl methane sulphonate, or MNNG, formed colonies in soft agar.

Colonies were removed from the soft agar and serially propagated to a cell density of 10^{6-3} . The ability of these cells to grow in soft agar was correlated with tumour formation in the nude (*nu/nu*) mouse (Table 2). Preliminary chromosome analysis confirmed that all cell lines examined had a human chromosomal constitution with a diploid or near diploid stem line. G-band analysis demonstrated that some lines, despite their normal diploid chromosome number, contained numerical deviations or abnormal chromosomes. C-band analysis revealed other alterations such as pericentric inversions and chromosomes with large areas of heterochromatin or abnormal chromosomes with more than one centromere. These transformed cells were injected into the subscapular region of the *nu/nu* mice previously irradiated with 450 rad (ref. 15). The bleb regressed between 24 and 96 h depending on the size of the inoculum. Subcutaneous tumours appeared in the subscapular areas 18–25 d after injection of the cell mass. After 4–6 weeks, the tumours (0.8–1.5 cm) were surgically removed, part was examined histopathologically and the rest transplanted into other mice. The tumours were composed of multiple vacuolated cell types predominantly a syncytial network with eosinophilic cytoplasm and ovoid basophilic nuclei. There

were also foci of more centrally very vacuolated basophilic staining cells with central or peripheral nuclei (Fig. 1). The presence of these cells composing the foci in the tumour mass is consistent with presence of proliferating exogenous cells producing the tumour mass. The tumours were classified as undifferentiated mesenchymal tumours, and the tumour incidence was recorded (Table 2). To date, all cell lines derived from agar injected into nude mice produce tumours. Tumours have been serially subpassaged $3 \times$ to date. Palpable growths formed in 4 weeks and reexamination of these growths confirmed their mesenchymal origin.

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Resorbing bone is chemotactic for monocytes

MONOCYTES are often found adjacent to bone-resorbing surfaces. Their function at these sites is unknown, although they have been shown *in vitro* to have the ability to resorb bone^{1,2}. There is also strong circumstantial evidence that circulating monocytes are precursors for the major bone-resorbing cell, the osteoclast^{3–7}. The mechanism by which circulating monocytes are attracted to sites of bone remodelling are unknown. We have examined the products of resorbing bone for chemotactic effects on circulating human monocytes and found that organ cultures of resorbing fetal rat long bones produced factors which were chemotactic for human peripheral blood monocytes, but not for neutrophils or lymphocytes. The chemotactic effect of resorbing bones occurred independent of the humoral mediator of bone resorption. The mechanisms by which monocytes are attracted to bone surfaces may be of fundamental importance to our understanding of bone cell dynamics and bone resorption.

The source of bone used in these experiments was the 19-d fetal rat radius and ulna, which had been previously labelled with ⁴⁵Ca as described previously^{8,9}. These bones resorb in organ culture in response to parathyroid hormone (PTH) and other stimulators of bone resorption^{8,9}. Bones were cultured

Fig. 1 A serial section of a tumour formed from proliferating aflatoxin B₁ treated cell populations in the nude mouse. The tumour was excised after 6 weeks in the mouse, fixed in glutaraldehyde and stained with haematoxylin and eosin. This tissue section reveals that tumour vascularisation has taken place. $\times 120$.

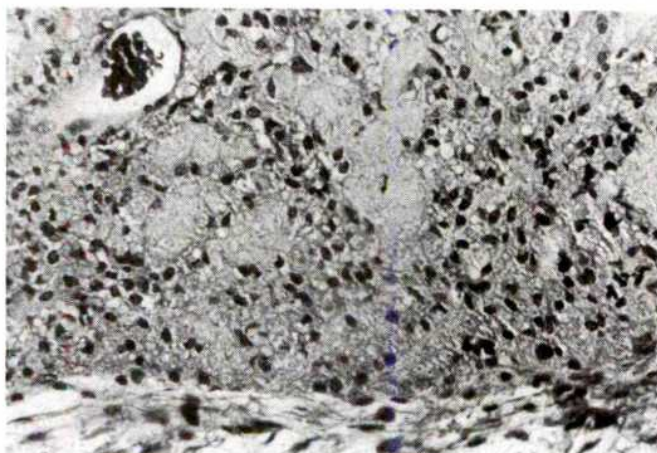


Table 1 Chemotactic responses of human monocytes and neutrophils to resorbing bone, using the Boyden chamber technique

Chemotactic sample	Bone resorption* (% ^{45}Ca release)			Monocyte chemotactic activity†		Neutrophil chemotactic activity†
	Expt A	Expt B	Expt C	Expt A	Expt B	Expt C
Media from PTH (400 ng ml $^{-1}$)-treated bones	65 ± 10‡	94 ± 3‡	83 ± 2‡	165 ± 15‡	61 ± 9‡	16 ± 1
Media from paired bones cultured in control media	27 ± 0.3	59 ± 7	33 ± 4	83 ± 6	27 ± 3	19 ± 2
Control media				86 ± 10	41 ± 5	29 ± 2
Control media + PTH (400 ng ml $^{-1}$)				94 ± 13		
Bacterial factor				140 ± 13‡	69 ± 14‡	102 ± 4‡
Leukotactic factor from zymosan-activated serum					118 ± 9‡	116 ± 6‡

Experiments A and B were carried out using polycarbonate filters (pore size 5 μm) and experiment C with cellulose acetate filters (pore size 0.65 μm). Bones were cultured in BGJ medium supplemented with bovine serum albumin (2 mg ml $^{-1}$). In experiments A and B the mononuclear cell concentration added to the upper compartment of the Boyden chambers was 5×10^6 cells ml $^{-1}$. In experiment C, the starting neutrophil concentration was 2.5×10^6 cells ml $^{-1}$. Bovine PTH (400 ng ml $^{-1}$) was provided by G. Auerbach; $1\alpha, 25(\text{OH})_2 \text{D}$ (10^{-8} M) was provided by Hoffman-LaRoche; PGE_2 (10^{-7} M) was from Upjohn.

* Values are means $\pm$ s.e.m. for 4 pairs of bones.

† Chemotactic activity is expressed as the average number of monocytes or neutrophils into the filter per high power field ($\pm$ s.e.m.).

‡ Significantly greater than control media ($P < 0.05$).

for 120 h in BGJ medium (Gibco) supplemented with bovine serum albumin (2 mg ml $^{-1}$) or 5% heat-inactivated fetal calf serum. PTH and other stimulators of bone resorption were added to culture dishes containing two long bones in 0.5 ml medium. Corresponding paired bones from the opposite limb were cultured in control media. Bone resorption was quantified by measuring the percentage of total ^{45}Ca released from the two bones into the medium during the culture period. Media taken from the stimulated and control bones were tested as potential attractants for human monocytes using both the Boyden chamber apparatus¹⁰ and the technique of monocyte chemotaxis under agarose¹¹.

Mononuclear cells were obtained from normal platelet-pheresis donations from the Connecticut Red Cross, Farmington¹. The leukocyte-rich buffy coat was separated from red cells and platelets by adding 5 ml 6% dextran and allowing the red cells to sediment by gravity. The buffy coat contained about 85% lymphocytes and 15% monocytes. Few polymorphonuclear leukocytes were present. The cells were suspended in BGJ medium at a concentration of 2.5×10^6 cells ml $^{-1}$ and added to the upper compartments of modified Boyden chambers¹⁰. The Boyden chambers were incubated in an atmosphere of 5% CO_2 and air at 37 °C for 4 h, after which the filters were removed, fixed, stained with haematoxylin, and examined by light microscopy (250 $\times$ magnification). Two types of Micropore filters were used, polycarbonate filters with uniform diameter linear pores of 5 μm diameter (Nucleopore) and nitrocellulose filters which have convoluted pores of 8 μm diameter (Schleicher and Schuell). Chemotactic activity was expressed as the number of cells which migrated into the filter in six random microscopic fields.

Table 1 shows the relative migration of mononuclear cells from the upper compartment of the Boyden chambers towards media from control or PTH-treated bones in the lower compartment. PTH in maximal doses caused more than 60% bone resorption as indicated by ^{45}Ca release. Media taken from the PTH-resorbed bones clearly attracted mononuclear cells into the membrane filters compared with media taken from the control bones and control media (Table 1). This effect was not due to PTH because when PTH-containing media which had not been cultured with bone was added to the lower compartment, there was no chemotactic effect (Table 1). In another experiment, PTH was added to media taken from bones cultured in control media, but again there was no increased chemotactic effect compared with control media alone, or control media cultured with bones for 120 h (data not shown).

The chemotactic activity of the resorbed bones was similar to that of two known powerful chemotactic agents, the leukotactic fractions obtained from zymosan-activated serum and the chemotactic factor produced by *E. coli*. Bacterial factor was obtained from overnight culture fluid of rapidly replicating *E. coli* cultures (WHO strain 16). The bacteria were removed by centrifugation and the factor was diluted 1/5 before use. Leukotactic fragments were obtained from serum by incubation with 10 mg zymosan per ml of serum at 37 °C for 1 h. When media from PTH-resorbed bones was added to the upper compartment or to both upper and lower compartments, there was no increase in movement of cells into the filters. Thus, increased movement of human monocytes into the membrane filters in response to resorbed bone media could not be ascribed to increased random cell migration.

The cells which were placed in the upper compartment of the Boyden apparatus at the beginning of the chemotaxis experiments were predominantly lymphocytes (85%). There were a smaller number of monocytes (15%) and polymorphonuclear leukocytes (<1%) (Fig. 1a). On the underside of the filter, only monocytes were observed (Fig. 1b). Thus, these experiments show that the chemotactic effect was directed towards monocytes and not lymphocytes or polymorphonuclear leukocytes. The absence of any effect on polymorphonuclear leukocytes

Table 2 Effect of different mediators of bone resorption on monocyte chemotaxis in Boyden chambers, using nitrocellulose filters with a pore size of 8 μm

Chemotactic sample	Bone resorption* (% ^{45}Ca release)	Chemotactic activity†
PTH (400 ng ml $^{-1}$) bone media	45 ± 8‡	48 ± 2‡
PGE_2 (10^{-7} M) bone media	32 ± 2‡	38 ± 2‡
$1\alpha, 25(\text{OH})_2 \text{D}$ (10^{-8} M) bone media	62 ± 4‡	55 ± 3‡
Control bone media	22 ± 1	32 ± 2
Dead bone media	14 ± 0.4	29 ± 2
Bacterial factor		52 ± 3‡

* Values are means $\pm$ s.e.m. for 6 pairs of bones.

† Chemotactic activity is expressed as the mean number of monocytes ($\pm$ s.e.m.) migrating into the filter per high power field (250 $\times$).

‡ Significantly greater than control bone sample ($P < 0.05$).

was confirmed by adding polymorphonuclear leucocytes alone to the upper compartment of the apparatus with resorbed bone media in the lower compartment. Although this PTH-treated bone media was actively chemotactic for monocytes, there was no increased chemotactic effect on polymorphonuclear leucocytes.

In another experiment, bones were incubated with two other humoral stimulators of bone resorption, 1,25-dihydrocholecalciferol and prostaglandin E₂ (Table 2). Media taken from these resorbed bones were also chemotactic for monocytes. There was a correlation between the extent of bone resorption and the chemotactic activity (Table 2). Thus, monocytes are attracted by media taken from resorbed bones independent of the humoral mediator of bone resorption.

The chemotaxis of monocytes towards resorbed bone products was also demonstrated using the technique of chemotaxis under agarose¹¹. In this method the cells migrate under semi-solid media in a tissue culture dish towards the adjacent chemotactic factor. A 10- μ l aliquot of cell suspension (containing 10⁶ cells) was placed in the centre well and four 19-d foetal rat radii and ulnae were placed in each outer well. In some dishes, PTH was added to the agarose-containing medium to stimulate the bones to resorb. Control dishes contained killed bones or unstimulated live bones. At the end of an 18-h incubation period in 5% CO₂ and air at 37°C, the cells were fixed with absolute methanol followed by formalin, and stained with Wright's stain. Migration was quantified by

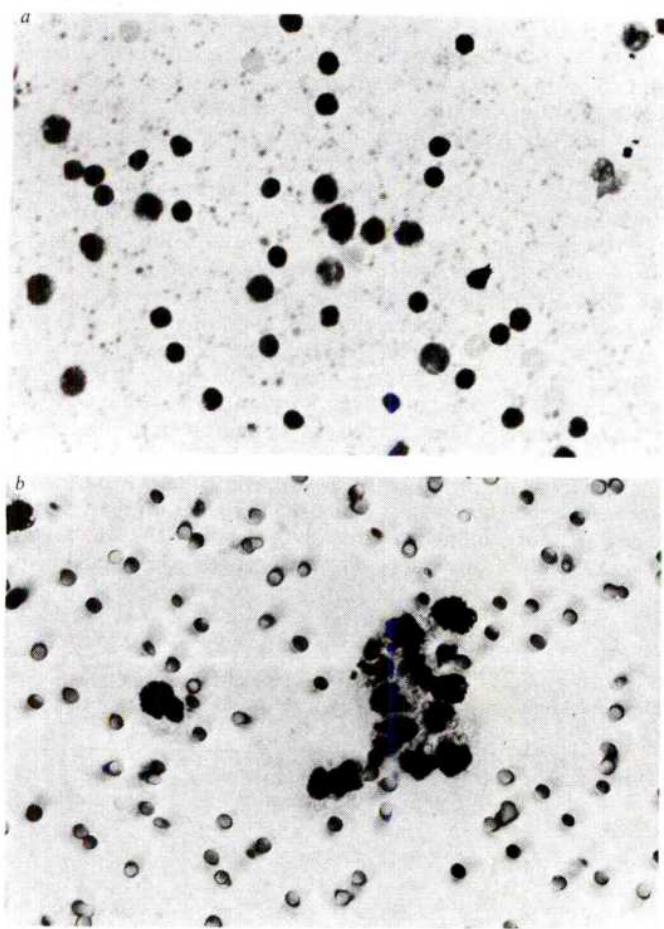


Fig. 1 *a*, Wright's stain of human leukocytes applied to upper compartment of Boyden chamber apparatus (250 $\times$). These cells were predominantly lymphocytes with a small number of monocytes and polymorphonuclear leukocytes. *b*, Wright's stain of cells which migrated through the membrane filter in response to resorbed bone products (250 $\times$). All of the cells observed were monocytes in spite of the mixed cell population added to the opposite (upper) compartment. The small circles are the pores in the polycarbonate filter.

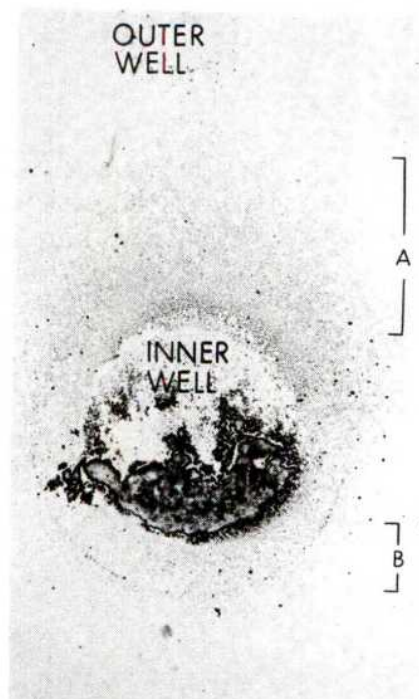


Fig. 2 Pattern of differential migration of human mononuclear cells in response to resorbing bones using the method of chemotaxis under agarose¹¹. Resorbing bones were in the outer well and the leukocytes (10⁶ cells in 10 μ l) were placed in the inner well. PTH (400 ng/ml) was added to the agarose medium. Distance A is the distance the cells migrated from the edge of the inner well towards the resorbing bones. Distance B represents spontaneous migration. The cells which migrated toward the bones in the outer well were monocytes.

projecting the pattern on to a white background and measuring the linear distance the cells had migrated toward the bones. In those dishes in which PTH was present, the bones were visibly resorbed and there was linear movement of the cells towards the wells containing the bones (Fig. 2). There was no elliptical ring of cells in dishes without PTH or in dishes which contained dead bones. The front advancing towards the resorbed bones comprised over 95% monocytes by light microscopic appearance.

The chemical nature of the factor or factors released by bone which is chemotactic for monocytes awaits further study. Small molecular weight factors such as calcium cannot account for these chemotactic effects since medium taken from PTH-treated bones did not lose chemotactic activity after dialysis. It is more likely that a fragment or product of bone matrix digestion is responsible. Postlewaite and Kang¹² have found that collagen-peptide fragments of chick and human skin collagen obtained by digestion with cyanogen bromide and pepsin or collagenase were chemotactic for monocytes but not for polymorphonuclear leukocytes. However, skin collagen differs chemically from bone collagen and whether similar chemotactic peptides are released during the bone resorption process is unknown.

The attraction of monocytes to bone resorbing margins may be of both physiological and pathological importance. Mononuclear cells are often found adjacent to bone-resorbing surfaces. Their role *in vivo* is unknown but it is possible that they participate in the bone-resorbing process because they are capable of resorbing bone *in vitro*^{1,2}. In addition, there is strong circumstantial evidence that osteoclasts, the major bone-resorbing cells, are derived from circulating mononuclear cells³⁻⁷. Thus, whether monocytes are precursors of osteoclasts or are bone-resorbing cells themselves, or both, the control of their movement to active bone margins may be a process of fundamental importance in bone resorption.

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Loss of immunoreactivity in long-term bone marrow culture

WE have developed a long-term bone marrow culture system in which proliferation and differentiation of apparently functionally normal haemopoietic stem cells (CFU-S) can be sustained *in vitro* for several months¹⁻⁸. We now find that genetic resistance^{9,10} or host-versus-graft (H-v-G) reaction, the (possibly) related natural killer (NK) cell activity¹¹ and the ability of cultured cells to reconstitute irradiated allogeneic (non-compatible) recipients (with the subsequent development of G-v-H disease¹²) are all absent from such cultured cells.

The maintenance of stem cells *in vitro* seems to depend on prior establishment of populations of marrow derived adherent cells (including endothelial cells, large, fat-containing cells and macrophages) which we speculate provide a suitable microenvironment necessary for sustained haemopoiesis^{2,3}. When such adherent layers are seeded with freshly isolated syngeneic or allogeneic marrow⁴, the stem cells migrate into the adherent layer and undergo extensive self-renewal, liberating cells into the growth medium⁵. Such cells can be collected and assayed at weekly intervals, and allow monitoring of events occurring in the cultures. Concomitantly with self-renewal, stem cells undergo differentiation to a variety of progenitor cells, including CFU-C (granulocyte/macrophage progenitors) and CFU-M (megakaryocyte precursors)^{1,6,7}. While subsequent development of BFU-E into mature erythroid cells does not occur in the culture system, it maintains maturation of CFU-C and CFU-M into mature granulocytes/macrophages and megakaryocytes respectively^{1,7}. No B- or T-lymphocytes are detected after the first week, although recent work indicates that a precursor cell, 'specific' for lymphopoiesis, is present even after several weeks⁸. This system has now been used to study the mechanisms underlying the phenomenon of bone marrow transplantation resistance.

When limited numbers of bone marrow cells from certain parental mice, for example C57BL/6, are injected into irradiated BDF₁ hybrids (C57BL/6 × DBA/2) a rejection mechanism ensures the killing of these cells within the first 1-2 days^{9,10}. This 'genetic resistance' is determined not by the H-2 antigens, but by non-codominantly inherited haemopoietic histocompatible

Table 1 Maintenance of stem cells (CFU-S) in allogeneic bone marrow chimaeras *in vitro*

Adherent layer	Cells inoculated		CFU-S maintenance	
	BDF ₁	C57BL/6	3DF ₁	C57BL/6
BDF ₁	1:1		>5 weeks	>5 weeks
	1:9		>5 weeks	>5 weeks
	9:1		>5 weeks	>5 weeks
	19:1		>5 weeks	>5 weeks
	1:19		>5 weeks	>5 weeks

Adherent layers established from BDF₁ femoral marrow¹ were inoculated after 3 weeks with 10 ml of growth medium containing different ratios of BDF₁:C57BL/6 bone marrow cells, to a final concentration of 10⁶ nucleated cells per ml. The cultures were then fed at weekly intervals¹ and the non-adherent cells assayed for CFU-S²¹ in BDF₁ and C57BL/6 recipients respectively. It had previously been established that at the cell numbers injected cultured C57BL/6 CFU-S would not form spleen colonies in BDF₁ mice and vice versa.

antigens (present on the surface of primitive haemopoietic cells) and *I*r genes, which determine the responsiveness. The cell-type involved in the rejection process seems to be bone-marrow derived, and shares properties in common with macrophages¹⁰. Previous work showed that the BDF₁-derived bone marrow adherent layers would support the growth of semi-allogeneic C57BL/6 parental stem cells⁴ and it was suggested that either the effector cell involved in genetic resistance was not present within the adherent layer population, or that the effector cell system had been 'swamped' by inoculation of excessive numbers of target cells. In more recent work, we have shown that a BDF₁ adherent layer will support the growth of very low numbers of semi-allogeneic stem cells. Using published procedures^{1,4} BDF₁ marrow cells were allowed to form an adherent layer for three weeks at which time all the growth medium was removed, and the adherent layer gently washed with growth medium. The cultures were then reconstituted with 10 ml of growth medium containing various ratios of BDF₁:C57BL/6 bone marrow cells, at a final concentration of 10⁶ cells per ml. The results (Table 1) show that irrespective of the cell combination used, there is always extensive proliferation and differentiation of both stem cell populations (in some experiments it has been possible to show proliferation of three or even four bone marrow stem cell chimaeras *in vitro*) and no evidence of rejection of the relatively low numbers of semi-allogeneic stem cells was observed.

When equal numbers of freshly isolated BDF₁ and C57BL/6 bone marrow cells were mixed, and allowed to establish an adherent layer before inoculation with DBA/2 bone marrow cells, stem cell proliferation was also observed—direct mixing of semi-allogeneic or allogeneic marrow cells did not inhibit the capacity of the adherent layer formed to support haemopoiesis, indicating also that the important milieu producing cells are not destroyed in such circumstances.

The clear association between effector cells involved in genetic resistance and natural killer (NK) cells¹¹ led us next to examine NK activity in these cultures (Table 2). The activity in freshly isolated marrow cells was appreciably lower than that seen with spleen cells, but culture of either spleen or bone marrow cells resulted in a rapid decline in activity—falling to levels not significantly above the background within 24 h following spleen cell culture. The loss of NK cell activity in the cultures, and the lack of response against allogeneic marrow grafts, supports the contention that similar effector cells are involved in both phenomena. Interestingly, resistance to certain leukaemia viruses and tumour cells has also been shown to be mediated by cells having characteristics in common with NK cells and host-versus-graft effector cells, suggesting that there may be a common effector cell^{11,14,15}. Yet, in studies with leukaemia virus infection of long-term bone marrow cultures, we have demonstrated the continued maintenance of resistance

Table 2 NK cell activity in normal or cultured spleen and bone marrow cells (BDF₁ mice)

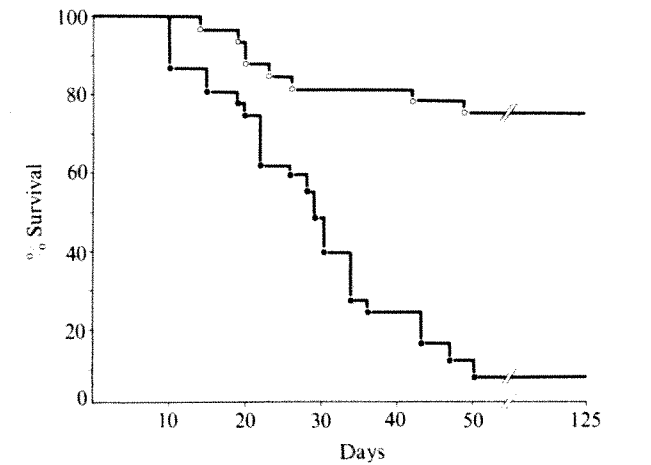
Effector: target cell ratio	Normal spleen Expt		Cultured spleen non-adherent cells 24 h Expt		Cultured spleen non-adherent + adherent cells 24 h Expt		Cultured spleen non-adherent + adherent cells 7 d	Normal bone marrow	Cultured bone marrow 7 d
	1	2	1	2	1	2			
100:1	27.0	30.7	2.8	1.9	1.1	2.3	6.8	12.0	3.3
50:1	13.7	16.7	1.9	1.0	1.2	1.1	3.2	6.5	0.8
25:1	9.4	7.7	1.7	1.1	1.1	1.5	6.0	3.4	0.0

Spleen cultures were established by inoculating 10⁸ spleen cells into culture flasks containing 10 ml of growth medium¹. Various times later the non-adherent cells were collected and assayed separately, or as a mixture, with the adherent cells for NK activity. Bone marrow cultures were established in the conventional way¹ and assayed 7 d after inoculation of the second bone marrow population. NK cell activity was measured by the cytotoxicity of spleen or bone marrow cells against ⁵¹Cr labelled K562 human CML in blast crisis target cells. 10⁴ K562 and varying numbers of effector (marrow or spleen) cells were incubated overnight and the ⁵¹Cr release from the cells measured according to published procedures²². Similar results were obtained when YAC-1 target cells were used.

in vitro^{16,17}. The cell type involved in this type of resistance, therefore, seems to be distinct from NK cell or H-v-G activity.

The possibility that cultured stem cells could be used to reconstitute the haemopoietic system of irradiated allogeneic mice, without the development of H-v-G disease, was indicated by our finding that T lymphopoiesis was not maintained in the cultures (since T lymphocytes are the effector cells involved in the H-v-G response⁸). Figure 1 demonstrates that 90% of BDF₁ mice, injected with normal C57BL/6 bone marrow cells, died within 70 d post reconstitution. On necropsy the mice had grossly enlarged spleens indicative of G-v-H disease^{12,19}. At this time only 20% of mice reconstituted with cultured C57BL/6 stem cells had died—with most of the deaths occurring in the first 4 weeks after reconstitution. At 120 d after reconstitution, some mice from this group were sacrificed, and the femoral marrow cells were assayed for CFU-S using irradiated BDF₁ and C57BL/6 mice as recipients. Spleen colonies developed only in the latter strain, showing that the cultured C57BL/6 marrow cells had indeed reconstituted the original BDF₁ recipients. Injection of cultured bone marrow cells, therefore, gives a situation analogous to that seen following reconstitution of irradiated mice with marrow cells which have been selectively depleted of T lymphocytes by immunological techniques^{18,20}.

Fig. 1 Survival of irradiated BDF₁ mice reconstituted with normal (●) or cultured (○) C57BL/6 bone marrow cells. C57BL/6 bone marrow adherent layers, established for three weeks, were 're-charged' with 10⁷ C57BL/6 bone marrow cells. The cultures were fed weekly by demi-depopulation. Two and three weeks later, the cells were collected and 5 × 10⁶–10⁷ cells containing approximately 750–1,500 haemopoietic stem cells or CFU-S²¹ were injected into irradiated (800 rad X rays) BDF₁ mice. Control mice received 5 × 10⁶–10⁷ freshly isolated (not cultured) C57BL/6 bone marrow—containing approximately equal numbers of CFU-S. Each group comprised 40 animals.



The significance of the present observations lies in the possibilities of establishing human cultures using allogeneic bone marrows and the potential of exploiting such cultures for the production of stem cells for marrow transplantation purposes (without the disadvantages of developing G-v-H disease). Furthermore, the absence of mature B, T and NK cells, in the murine system is allowing work on the genesis of these cells from the pluripotent haemopoietic stem cell.

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Hepatic glucocorticoid receptors and the H-2 locus

VARIOUS inbred strains of mice exhibit different degrees of susceptibility to both spontaneous and glucocorticoid-induced cleft palate^{1,2}, and specific genetic and biochemical factors that may be responsible for these differences have been identified. At the genetic level, variations at the H-2 locus have been associated with susceptibility to glucocorticoid-induced cleft palate^{3,4}, while at the biochemical level, evidence suggests that numbers and characteristics of glucocorticoid receptors in murine embryonic facial mesenchyme cells differ in the various

strains. Specifically, Salomon and Pratt showed that facial mesenchyme cells cultured from fetuses of the A/J strain (which is highly susceptible to glucocorticoid-induced cleft palate) contain twice as many glucocorticoid receptors as do such cells from C57BL/6J, a low susceptibility strain⁵. They also reported that the glucocorticoid receptor of the A/J strain has a lower affinity for dexamethasone ($K_D = 1.7 \times 10^{-8}$ M) than does that of the C57BL/6J strain ($K_D = 7.9 \times 10^{-9}$ M) (ref. 5). In an attempt to relate steroid receptor activity to *H-2* haplotype, Goldman *et al.* used ³H-cortisol and isoelectric focusing to study glucocorticoid receptors in facial mesenchyme cells of these and several other strains⁶. They concluded that "a product of a gene in or near the *H-2* locus seems to be the glucocorticoid receptor, the level of which must be increased for a congenital malformation to occur". Such conclusions must be questioned, however, because the experimental procedure required micro-electrofocusing of isolated protein-cortisol complex for 40 h. Receptor binding is reversible, and in the absence of free steroid, the half life of the receptor-cortisol complex is only about 1 h at 0 °C (ref. 7). It is unlikely that a peak of radioactivity detected by isoelectric focusing after 40 h represents binding to a specific receptor. Thus an association between *H-2* haplotype and glucocorticoid receptor activity has not been established definitively. However, the possibility that *H-2* might determine hormone receptor levels or characteristics is exciting. We have explored the relationship between *H-2* and glucocorticoid receptors by examining the level of specific glucocorticoid-binding activity in liver cytosols prepared from different mouse strains.

Liver is suitable for such studies because liver cytosol preparations tend to have a relatively stable glucocorticoid-binding activity^{8,9}, while unbound glucocorticoid receptor molecules undergo rapid, temperature-dependent inactivation in most other cell-free systems. The liver is also well characterised as a target of glucocorticoid action, and *H-2* is expressed in this organ¹⁰. Thus an effect of *H-2* haplotype on the amount or affinity of specific glucocorticoid binding should be observed in this tissue. We examined specific glucocorticoid binding in the two strains studied by Salomon and Pratt, A/J (high susceptibility) and C57BL/6J (low susceptibility), as well as in the two *H-2* congenic lines A.BY and B10.A.

Because glucocorticoid-receptor inactivation is greatly accelerated at elevated temperatures⁷, we took care to prepare and maintain the liver cytosol at 0 °C. Adult males were killed by decapitation. Livers were removed and chopped on glassine paper over dry ice, weighed and transferred to a chilled homogeniser. Four volumes of ice-cold buffer (10 mM Tris, pH 7.35, 0.1 mM EDTA) were added and the tissue was homogenised on ice with nine strokes of a motor-driven Teflon pestle. The homogenate was centrifuged at 35,000g for 30 min, the supernatant was removed and assayed for glucocorticoid-binding activity.

Figure 1 shows binding of ³H-TA to the macromolecular component of liver cytosol as a function of free steroid concentration for the A/J and C57 strains. Nonspecific binding (binding of ³H-triamcinolone acetonide (TA) in the presence of an excess of cold dexamethasone) is the same in both strains, whereas total binding is significantly higher in A/J. Specific binding (total less nonspecific, upper inset, Fig. 1) is about twice as high in A/J as it is in C57. Scatchard plots of the data (Fig. 1, lower inset) suggest a single class of high-affinity binding sites. The initial slopes, representing specific binding, are approximately equal, giving an apparent K_D of 1.8×10^{-8} M for both the A/J and C57 strains. Experimentally determined values for specific binding and apparent dissociation constants can be affected significantly by inactivation of the unbound receptor. Rates of receptor inactivation must be similar in cytosol preparations from the two strains before values for dissociation constants and specific binding can be usefully compared¹¹. Figure 2 shows the decline of specific binding activity observed when liver cytosol is incubated in the absence of free steroid for increasing periods. Rates of inactivation of unbound receptors

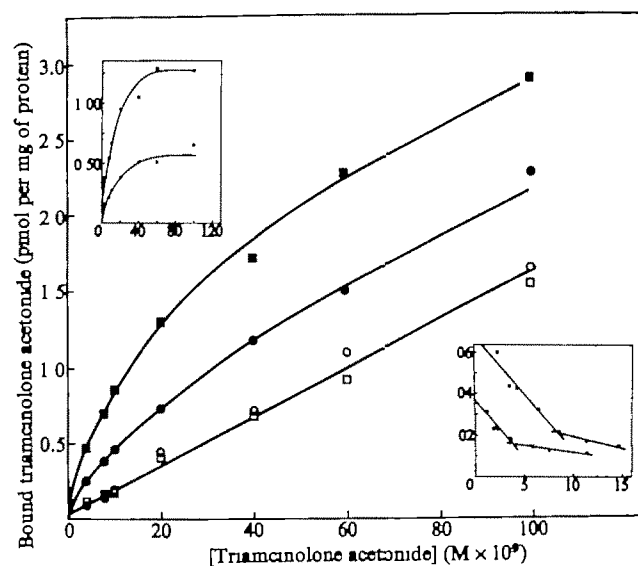


Fig. 1 Binding of ³H-TA presented as a function of free steroid concentration. Samples of 0.2 ml of liver cytosol were incubated in a total volume of 0.5 ml for 22 h at 0 °C with various concentrations of ³H-TA ($21.6 \text{ Ci mmol}^{-1}$) in the presence of vehicle or a high concentration (5×10^{-5} M) of competing, nonradioactive dexamethasone. The bound steroid was separated from the free compound by passage through columns (1 cm $\times$ 22 cm) of Sephadex G-25 with an elution buffer of 0.01 M Tris (pH 7.35), 0.04 M KCl. The columns were run at 4 °C. Fractions (1 ml) were collected and the macromolecular peak was identified by the presence of blue colour resulting from the addition of two drops of a solution of blue dextran to each sample immediately prior to application to the column. Each point represents the binding expressed as pmol bound per mg of protein. Binding in the presence of vehicle: $\blacksquare$, A/J; $\bullet$, C57BL/6J, binding in the presence of nonradioactive dexamethasone (nonspecific binding); $\square$, A/J; $\circ$, C57BL/6J. The upper inset shows specific binding (total minus nonspecific) for each strain. Bound TA (pmol per mg of protein) is plotted against unbound TA ($\text{M} \times 10^5$). The lower inset presents Scatchard plots of the binding in the presence of vehicle. The ratio of bound to unbound TA is plotted against bound TA ($\text{M} \times 10^{10}$).

are the same in both strains. These rates and the observed levels of glucocorticoid binding were not affected by dithiothreitol.

To determine whether or not differences in glucocorticoid-receptor activity observed in the two strains are mediated by the *H-2* locus, activities of glucocorticoid receptors in the two congenic lines, A.BY and B10.A, were determined. A.BY has the *H-2^b*-region of the C57 strain placed on the A genetic background, while B10.A has the C57 background, but the A/J *H-2^a*-region. The results are shown in Table 1. The mean specific binding capacities in the four strains are significantly different ($P < 0.002$), however the difference is due to genetic background ($P < 0.001$) and not *H-2* ($P = 0.99$). There is some possibility of an *H-2* $\times$ background interaction effect ($P = 0.10$), which in this case would mean that the effect of genetic background on levels of glucocorticoid receptors is more pronounced in the presence of the *H-2^b*-haplotype than in the presence of the *H-2^a*-haplotype.

Thus we have shown that (1) rates of glucocorticoid-receptor inactivation are the same in both A/J and C57BL/6J mice; (2) affinities of the receptors for TA are the same in both strains; (3) A/J has approximately twice as many glucocorticoid receptors as C57BL/6J, and (4) this difference is not determined by variations at the *H-2* locus.

It is interesting to compare our results with what is known about spontaneous and glucocorticoid-induced incidence of cleft palate in these strains. The incidence of cortisone-induced cleft palate in A/J is 100%, in B10.A, 81% and C57, 21% (ref. 4), which corresponds in rank order to the observed levels of

Table 1 Relationship of specific glucocorticoid-binding capacity to *H-2* and genetic background

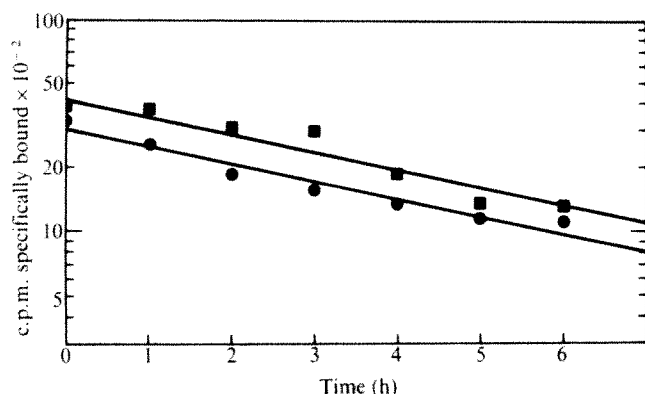
Background	Haplotype	
	<i>H-2</i> ^a	<i>H-2</i> ^b
A	A/J	A.BY
	1.29 ±0.11 (10)	1.49 ±0.16 (9)
C57Bl	B10.A	C57Bl
	0.99 ±0.10 (9)	0.83 ±0.09 (10)

Incubations (0.5 ml) containing liver cytosol and 6×10^{-8} M ³H-TA in the presence of vehicle or nonradioactive competing dexamethasone were maintained for 3 h at 0 °C. Specifically bound steroid was determined as outlined in the legend to Fig. 1. The numbers represent mean specific binding (pmol of ³H-TA bound per mg of cytosol protein) ± standard error for each of the four strains. Specific binding was determined in duplicate for each animal. The number of animals used to determine each mean is given in parentheses.

glucocorticoid receptors in these strains. The steroid-induced frequency of cleft palate in A.BY was not studied, but in our laboratory it has the highest spontaneous incidence of cleft palate of all four strains (19, 3, 0 and 0% for A.BY, A/J, B10.A and C57, respectively). Because A.BY also has the highest level of steroid receptors, glucocorticoid receptor levels may be an important determinant of susceptibility to spontaneous cleft palate in mice.

Although susceptibility to glucocorticoid-induced cleft palate has been shown to be partially controlled by *H-2* haplotype, these data demonstrate that the level of hepatic glucocorticoid receptors is determined by other genetic differences between the strains. Whether the factors controlling the levels of binding activity in liver reflect those that operate in the mesenchymal cells of the fetal palate is of course open to question. We observe the same difference in glucocorticoid binding capacity in liver as was seen by Salomon and Pratt in cultured fetal facial mesenchyme cells. This strongly suggests that the observed genetic control of receptor levels is not tissue specific. Our data, unlike theirs, do not suggest different affinity constants for the receptors in the two strains. Differences in binding capacity between the strains could arise from differences in the rates of receptor synthesis or degradation, or as has been suggested¹²⁻¹⁴, differences in rates of receptor activation and/or inactivation. Given the equivalent rates of inactivation presented in Fig. 2, the latter does not seem to be likely in this case.

Fig. 2 Unbound-receptor inactivation at 0 °C. Liver cytosol was incubated at 0 °C in the absence of labelled steroid for various times, after which saturating levels (6×10^{-8} M) of ³H-TA were added in both the presence and absence of an excess of cold dexamethasone. Specifically bound steroid was determined as described in the legend to Fig. 1. ■, A/J; ○, C57.



Although *H-2* is not the locus of prime importance in determining levels of hepatic glucocorticoid receptors in these strains, it may still be possible, using recombinant inbred lines, to identify specific loci which do regulate levels of glucocorticoid receptors. It may be particularly informative if lines with high levels of receptors and low susceptibility to glucocorticoid-induced cleft palate could be found.

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Effect of L-triiodothyronine on (–)³H-dihydroalprenolol binding and cyclic AMP response to (–)adrenaline in cultured heart cells

MANY physiological responses of the cardiovascular system to thyroid hormone seem to reflect altered sympathetic activity. The effects of thyroid hormones on the heart are very similar to those induced by catecholamines and the possible interrelationship of these substances on myocardial function has been explained by an interaction at various levels. These include effects on altering catecholamine release from the sympathetic nervous system^{1,2}, and modifying plasma catecholamine concentrations^{3,4}, as well as tissue catecholamine content⁵ and responses⁶⁻⁸. Although several investigators have attempted to define an action of thyroid hormone and its possible role in potentiating the catecholamine response, the mechanism responsible for this modification has not been clarified. We have reported previously that physiological concentrations of L-triiodothyronine (T₃) stimulate the rate of glucose metabolism in cultured heart cells prepared from newborn rats⁹. We now demonstrate here that cultured heart cells retain the characteristic of response to β-adrenergic stimulation and that this response seems to be influenced by T₃.

The heart cells were prepared as described previously^{9,10}. The trypsinised cells were inoculated into 25-cm² plastic T-flasks (Falcon) at densities of approximately 200,000 cells cm⁻² and then grown in Ham's medium (Gibco) supplemented with 10% fetal calf serum (FCS) at 37 °C (95% air, 5% CO₂).

Catecholamine action seems to involve activation of adenylyl cyclase, which then leads to an increase in the intracellular concentration of cyclic AMP¹¹. Therefore we examined the cyclic AMP response to (–)adrenaline and the inhibitory effect

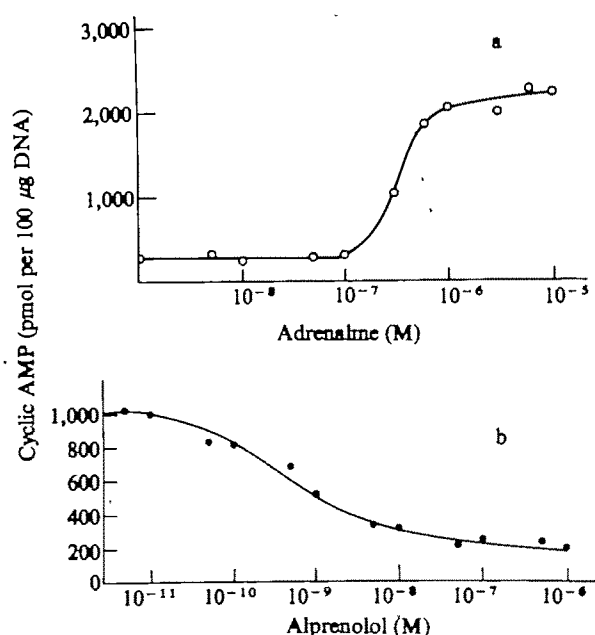


Fig. 1 Dose-response relationship of *a*, (-)-adrenaline stimulation of cyclic AMP production and *b*, (-)-alprenolol inhibition of the (-)-adrenaline-induced cyclic AMP response in the cultured heart cell. *a*, After 5 d growth of the culture, the culture flasks were partially submerged in a water bath at 37 °C for 2 min, to enable there to be precise temperature control during the experiment. The upper surface of the flasks was then opened with a haemostat. Various concentrations of (-)-adrenaline bitartrate (Sigma) dissolved in Ham's F-10 medium was added to the culture media. After a 2-min incubation, the media were aspirated and 1 ml of 10% ice-chilled trichloroacetic acid (TCA) was added. The cellular material was then removed using a rubber policeman at 4 °C and the samples were centrifuged at 2,000g for 15 min. The cell TCA precipitates were analysed for DNA by the method of Burton²⁰, and the supernatant was used for cyclic AMP assay by a modification of competitive binding method of Gilman²¹. The concentration of (-)-adrenaline which induced a half-maximal cyclic AMP response was 0.2 µM; above a concentration of 1 µM, (-)-adrenaline induced a maximal cyclic AMP response. *b*, Blocking effect of (-)-alprenolol on (-)-adrenaline-induced cyclic AMP production in a dose-related fashion. Various concentrations of (-)-alprenolol were added to the culture for 30 min before the addition of (-)-adrenaline (1 µM). The concentration of (-)-alprenolol which gave half-maximal inhibition was approximately 0.27 µM.

of the catecholamine antagonist, (-)-alprenolol, on the heart cell cultures. (-)-Adrenaline bitartrate at 1 µM, induced a sixfold increase in intracellular cyclic AMP within 2 min of incubation, which subsequently declined to a plateau higher than the basal level for up to 30 min. The intracellular cyclic AMP in cultured heart cells responds to (-)-adrenaline in a dose-related fashion; at concentrations greater than 1 µM, (-)-adrenaline induces a maximal response (Fig. 1*a*). This response of cyclic AMP can be antagonised effectively by (-)-alprenolol, which is a potent β -adrenergic antagonist, also in a dose-dependent fashion (Fig. 1*b*). The effect of preincubation of the cells with various concentrations of (-)-alprenolol for 30 min before induction of cyclic AMP by (-)-adrenaline was studied. (-)-Alprenolol at concentrations greater than 1 µM completely inhibited the adrenaline-induced cyclic AMP response, indicating that the cultured heart cells possess a biologically responsive β -adrenergic receptor.

It is generally felt that the physiological action of catecholamine agonists which result in an increase of intracellular cyclic AMP are mediated by a β -adrenergic receptor¹². Binding sites with the specificity of β -adrenergic receptor determined by (-)-³H-dihydroalprenolol binding have been identified in avian

and amphibian erythrocytes¹³, human lymphocytes¹⁴, and canine myocardium¹⁵. We have also demonstrated that specific binding of (-)-³H-dihydroalprenolol to cultured heart cells was very rapid at 37 °C, attaining equilibrium within 5 min. This binding can also be displaced equally rapidly by catecholamine agonists and antagonists.

We have examined the influence of T_3 on β -adrenergic responses, by measuring (-)-³H-dihydroalprenolol binding and the cyclic AMP response to (-)-adrenaline. The heart cells were grown first in regular growth medium and then this was replaced with Ham's F-10 medium supplemented with 10% hypothyroid calf serum. The cells remained in the second medium for 24 h, in order to deplete thyroid hormone. The culture medium was then

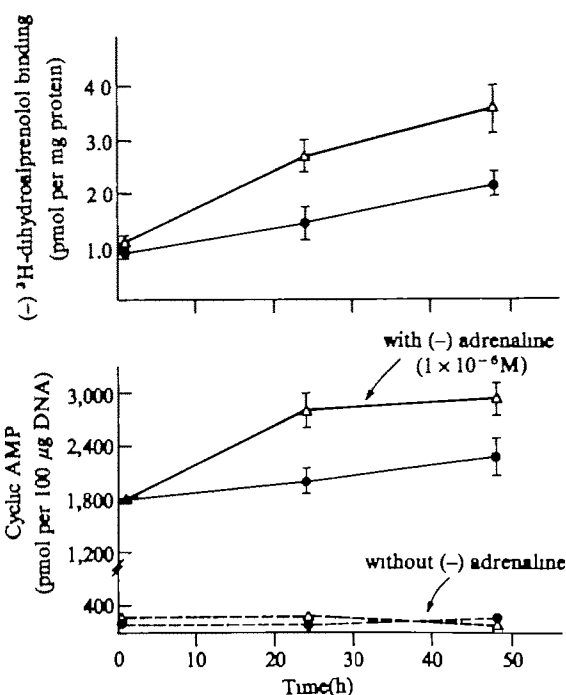


Fig. 2 Effect of L-triiodothyronine on (-)-³H-dihydroalprenolol binding (upper), cyclic AMP response (-)-adrenaline (lower). The heart cell cultures were prepared as described in the text. T_3 at 5 nM was added to one group of flasks. At the times indicated, (-)-³H-dihydroalprenolol binding and the cyclic AMP response to (-)-adrenaline (1 µM) were measured. For study of (-)-³H-dihydroalprenolol binding, the cells were scraped off the flasks with a rubber policeman and then washed twice with 10 ml of 0.145 M NaCl by centrifugation. The cells were suspended in 50 mM Tris-HCl buffer (pH 7.4 at 25 °C) containing 10 mM MgCl₂. Binding studies were carried out by incubating the cells (equivalent 12–18 µg of cell protein per tube) at 37 °C with 80 nM of (-)-³H-dihydroalprenolol (NEN, 32.5 Ci mmol⁻¹) in a total volume of 150 µl of incubation buffer. This concentration of (-)-³H-dihydroalprenolol gives maximal binding in the culture heart cells. The incubation was terminated by addition of 2 ml of ice-chilled buffer solution, followed by rapid vacuum filtration through Whatman GF/C glass fibre filters with a Millipore sampling manifold. The filters were then washed with 12 ml of ice-chilled buffer and were dried overnight at room temperature. The samples were counted with a Beckman liquid scintillation spectrometer with Aquasol solution (NEN). Nonspecific binding was determined by measuring the amount of radioactivity retained in the presence of a 10,000-fold excess of non-radiolabelled (-)-alprenolol (Sigma). The specific binding illustrated represented the total minus the nonspecific binding. An aliquot of cell suspension from each experiment was analysed for DNA content by the method of Burton²⁰ and cellular protein by the method of Lowry *et al.*²². The cellular protein-DNA ratio was not affected by thyroid hormone during the period of experiments. After incubation for 24 h, T_3 induced a significant increase in the (-)-³H-dihydroalprenolol binding and in the cyclic AMP response to (-)-adrenaline. Δ , With T_3 (5×10^{-9} M); $\bullet$, without T_3 .

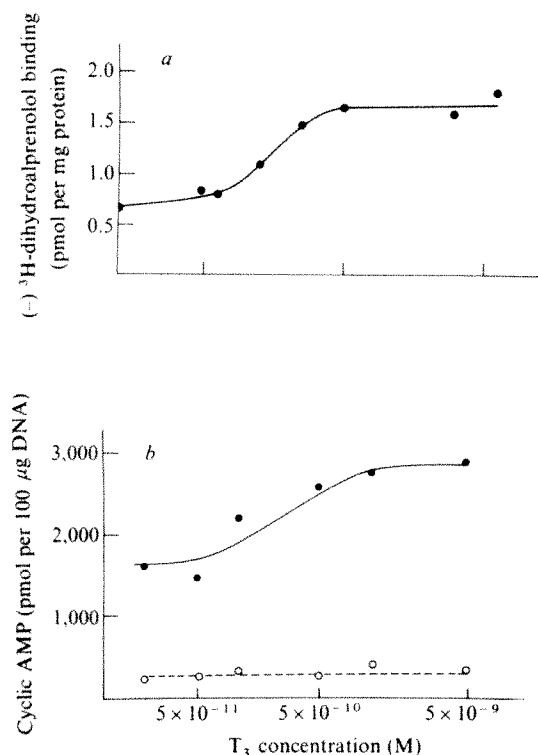


Fig. 3 Effect of T₃ on *a*, (-)-³H-dihydroalprenolol binding and *b*, (-)-adrenaline-induced cyclic AMP response. The cultured heart cells were incubated with medium containing various concentrations of T₃ for 24 h and the (-)-³H-dihydroalprenolol binding (*a*) and cyclic AMP response to 1 µM of (-)-adrenaline (*b*), were studied simultaneously. T₃ affected both responses in a dose-related fashion. T₃ alone seemed to have no effect on the intracellular cyclic AMP level. ●, With 1 × 10⁻⁶ M (-)-adrenaline; ○, without (-)-adrenaline.

altered a third time, by adding 5 nM of T₃ to one group of flasks. (This concentration of T₃ has been shown to induce the maximal rate of glucose metabolism in cultured heart cells⁹ and growth hormone production in cultured GH₁ cell system¹⁶, a well established cell-culture system which is responsive to physiological concentration of thyroid hormone.) After 24 h of incubation, T₃ induced a significant increase of (-)-³H-dihydroalprenolol binding on culture heart cells (Fig. 2). The cyclic AMP response to (-)-adrenaline was similarly increased by T₃.

We next examined the influence of T₃ concentrations on (-)-³H-dihydroalprenolol binding and the cyclic AMP response to 1 µM (-)-adrenaline. As shown in Fig. 3*a*, (-)-³H-dihydroalprenolol binding was increased by thyroid hormone in a dose-dependent fashion. The cyclic AMP response to (-)-adrenaline was also enhanced by thyroid hormone in an identical fashion (Fig. 3*b*). The concentration of T₃ (0.3 nM) which resulted in a half-maximal stimulation of cyclic AMP by (-)-adrenaline and a half-maximal increase in (-)-³H-dihydroalprenolol binding is virtually identical. Cells incubated with 5 nM of T₃ showed almost a twofold increase in (-)-³H-dihydroalprenolol binding.

L-Triiodothyronine does not affect cell growth as reflected by total cellular DNA or protein content of the cultured cells⁹. The increase in (-)-³H-dihydroalprenolol binding therefore seems to be influenced selectively by T₃ and to be independent of total cell protein. The possibility cannot yet be excluded that T₃ may induce an alteration in membrane structure which cannot be measured in this study¹⁹. Martin and co-workers reported the existence of a β-adrenergic receptor in 13-d-old fetal rat hearts¹⁷. Here we have demonstrated that the β-adrenergic response of cardiac tissue can be maintained in dispersed cultured condition and can be influenced by thyroid hormone in a dose-related fashion. Thyroid hormone has recently been

reported to increase the β-adrenergic receptor in rat myocardium¹⁸. The exact mechanism by which thyroid hormone regulates the β-adrenergic response requires further investigation.

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Possible plasticity in the rat superior colliculus

THE potential for plasticity of the developing mammalian visual system has been the subject of several investigations^{1,2}. I have now explored the plastic relationship between different sensory modalities. The rodent superior colliculus was chosen as the model as it receives overlapping visual, somatic and auditory inputs^{3,4}. Electrophysiological comparison of dark-reared and normal rats suggests that the rat's superior colliculus is susceptible to effects of sensory deprivation during its postnatal development.

It has been shown in the mouse³ and the hamster⁴ that many neurones in the intermediate and deep layers of the optic tectum receive nonvisual sensory inputs. They respond either to somatic stimuli from fur or whiskers on the contralateral half of the body or to complex sounds from the contralateral side. Some of these cells also receive visual inputs and thus have a bimodal response; more rarely, trimodal cells responding to all three sensory modalities are also encountered. Further, for cells in any one electrode penetration that is nearly normal to the collicular surface, there is general topographical correlation between all the visual, somatic and auditory receptive fields.

In my study using black-hooded rats, I have compared normally reared (12 h light/dark cycle) and dark-reared rats for the relative abundance of the different types of single units in the superior colliculus. I adopted a double-blind procedure, in which the rearing condition of each animal was not revealed to me until I had completed recordings on all the rats. Twenty-nine rats (15 normal and 14 dark-reared), all approximately 20 weeks old and weighing about 300 g, were used in random order. Light deprivation was achieved by rearing the animals in a dark room and the only light they could have received until the time of recording was a few minutes of a red photographic light (<0.1 lm m⁻²) during cleaning of cages.

Table 1 Distribution of sensory cell types in the deeper layers of the superior colliculus of normal and light-deprived rats

	Visual	Somatic	Auditory	Visual/ somatic	Bimodal neurons Visual/ auditory	Somatic/ auditory	Trimodal neurons (visual/ somatic/ auditory)	Total
Normal rats	104 (46.2%)	85 (37.8%)	9 (4%)	18 (8%)	3 (1.3%)	3 (1.3%)	3 (1.3%)	225 (100%)
Light-deprived rats	56 (24.5%)	140 (61.1%)	24 (10.5%)	2 (0.9%)	0	7 (3.1%)	0	229 (100%)

The basic operating and recording techniques were similar to those used by Tiao and Blakemore⁴ for the hamster. Glass-coated tungsten microelectrodes with a tip length of 15–20 µm were used and single units were identified by the usual criteria⁵. As the superficial layers of the tectum are densely packed with visual cells even in the dark-reared rats, I have attempted to include in my count cells from intermediate and deep layers only. The occurrence of changes⁴ in the response properties to visual stimuli during an electrode penetration and prior histology indicated the approximate limit to the superficial layers to be 350 µm below the collicular surface. However, all cells encountered below 200 µm were included in the count. The surface of the superior colliculus was identified during the nearly surface-normal penetration by a characteristic change in the background noise, which responded to visual stimuli in the correct part of the visual field with a brisk hiss. The penetration was always continued to a depth of 2,200–2,500 µm, which was at least 500 µm beyond the last cell responding to a sensory stimulus. Histological reconstruction of the electrode tracks in some of the animals confirmed the reliability of our electrophysiological criteria for identifying the superior colliculus. All recordings were restricted to the parts of the superior colliculus whose visual field representation was between 40° and 80° from the midline, with the blind axis monitored at 70°. This was necessary because, towards the caudal end of the colliculus, representing the temporal end of the visual field, there is greater nonvisual input even in the normal rat, and towards the rostral end of the colliculus, representing the nasal end of the visual field, there is greater visual input even in the dark-reared rat. When tested ophthalmologically before recording, none of the rats used showed any obvious difference from the controls.

Table 1 shows the pooled results from 42 penetrations in 15 normal rats and 40 penetrations in 14 dark-reared rats. It can be readily seen that the ratio of nonvisual cells to visual ones has increased markedly in the light-deprived animals. Further, there is an almost total absence of multimodal cells with visual inputs in these animals. The increase in the number of nonvisual cells can be either a relative one due to functional or anatomical dropping out of the visual terminals or may reflect an active take-over of collicular neurones by nonvisual sensory inputs. I have attempted to resolve this question by comparing the mean distribution of cell types per penetration between the two groups of rats. It can be seen from Table 2 that the mean number of cells in a single penetration having visual input has significantly (two-tailed test: $P < 0.001$) decreased in the dark-reared rats. Further, the mean number of cells with nonvisual sensory inputs

has significantly ($0.001 < P < 0.01$) increased, suggesting an active process. This absolute increase in the mean number of neurones responding to nonvisual stimuli also makes it less likely that the reduction in the number of visually driven cells is due to any shrinkage of these cells in the light-deprived rat. However, the contribution of any retinal effects of light deprivation⁶ to the reduction in the number of recordable visual cells is an open issue.

It may not be too speculative to suggest that, at birth, the sensory inputs to the superior colliculus from the different modalities are in a state of competition and in the absence of normal stimulation in one of these channels, the other modalities may come to influence a larger proportion of cells. It does not, however, follow that the discriminative ability of the somatic and auditory systems in the light-deprived rat are, compensatively, better than, or even as good as that in the normal rat. The postnatal visual input may have a directive role in the development of the normal properties of the nonvisual and multimodal neurones of the optic tectum. In its absence, the somatic and auditory systems may in fact be permanently impaired.

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Horizontal cells influence membrane potential of bipolar cells in the retina of the turtle

BIPOLAR CELL responses to illumination of the surround have been thought to be mediated by horizontal cells^{1,2}. However, it has been shown recently that the responses of bipolar cells to stimulation with an annulus of light are not suppressed by pharmacological blockade of horizontal cell activity, so alternative mechanisms for the surround effect have been proposed³. The present experiments were performed to establish whether bipolar cells are in fact driven by horizontal cells, and whether the properties of such hypothetical interaction can account for the surround responses of bipolar cells.

Two intracellular microelectrodes were used simultaneously: one to pass current through the membrane of a horizontal cell, and the other to record the membrane potential of an adjacent

Table 2 Mean distribution of cell types per electrode penetration in the superior colliculus of normal and light-deprived rats (± s.d.)

	Cells with visual inputs*	Cells with nonvisual sensory inputs†
Normal rats	3.0 ± 1.3	2.9 ± 1.9
Light-deprived rats	1.5 ± 1.0	4.3 ± 2.1

* Includes multimodal cells with visual inputs.

† Includes multimodal cells with nonvisual sensory inputs.

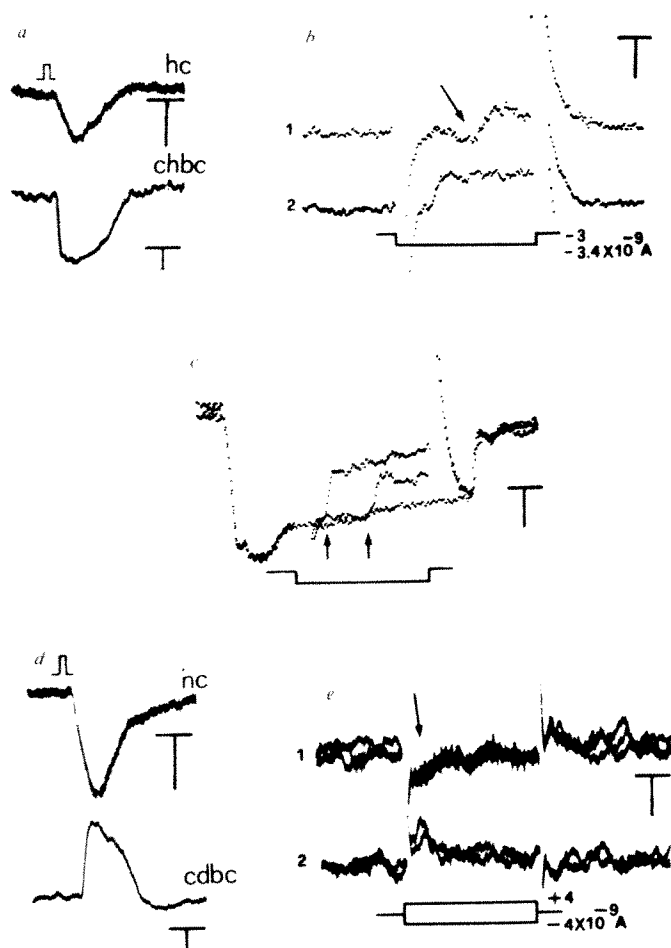


Fig. 1 Simultaneous recordings from L-type horizontal (hc) and bipolar cells. Bipolar cells were centre-hyperpolarising (c.h.b.c) in *a* and centre-depolarising in *d* and *e* (c.d.b.c). *a*, Photoreponses to brief illumination (25 ms, as indicated upper left) in the form of a spot, of 200 μm radius centred on the electrodes penetration site. The light source ($2 \times 10^5 \text{ erg cm}^{-2} \text{ s}^{-1}$, with wavelength between 400 and 800 μm) was attenuated by 3.6 log units. Note that the hc response is 25% of its maximal amplitude with bright light, while the c.h.b.c response has already reached the amplitude saturation level. *b*, Computer-averaged recordings from the same c.h.b.c as in *a*, in darkness. Each record represents the average of 8 sweeps. The lower line represents the duration of the horizontal cell artificial polarisation obtained with the current intensity indicated by the numbers at the right. The arrow in 1 points to the onset of the depolarising response of c.h.b.c as in *a*. *c*, Computer-averaged recordings from the same c.h.b.c as in *a*. Three superimposed averages of two photoresponses each, recorded without and with artificial horizontal cell hyperpolarisation, whose duration is indicated by the lower line. The arrows point to the onset of the c.h.b.c depolarising responses to current injections of the same intensities as in *b*. A spot of 500 μm radius was used for illumination. Duration of illumination is indicated approximately by the onset and the recovery phase of the c.h.b.s membrane potential. Light intensity as in *a*. *d*, Responses from a horizontal cell (hc) and a bipolar cell (c.d.b.c) to brief illumination (25 ms, as indicated at the upper left) in the form of a spot of 200 μm radius. Light attenuation was -3.6 log units. *e* 1, Superimposed recordings, from the same c.d.b.c as in *d*, of the hyperpolarising responses (arrow) to artificial hyperpolarisation of the horizontal cell; *e* 2, depolarising c.d.b.c responses to artificial depolarisation of horizontal cell. Duration of current injection is indicated by the lower line. Numbers to the right refer to the intensity of injected current. Time and voltage calibrations (100 ms, 5 mV) are indicated by the horizontal and vertical lines, respectively, shown as T symbols, at the right of each recording, from *a* to *e*.

bipolar cell. The methods for intracellular recording and light stimulation have been described elsewhere⁴.

Figure 1*a* shows the simultaneously recorded responses from an L-type horizontal cell and a centre-hyperpolarising bipolar

cell, identified according to criteria for turtle retina defined previously^{2,5}. The stimulating light, a spot of 200 μm radius, was centred midway between the electrodes, which were about 100 μm apart. When hyperpolarising current was passed through the horizontal cell membrane, a sustained depolarisation was recorded from the centre-hyperpolarising bipolar cells (Fig. 1*b*(1)). Increasing the current induced a larger response and reduced latency (Fig. 1*b*(2)). In this cell, the lowest detectable response was evoked by injecting $1.7 \times 10^{-9} \text{ A}$, which produced an estimated -1.7 to -3.4 mV change of horizontal cell membrane potential (assuming a horizontal cell input resistance of $1-2 \text{ M}\Omega$). Similar results were obtained during illumination (Fig. 1*c*), and the bipolar cell response to current injection disappeared when the electrode was withdrawn from the horizontal cell. On the other hand, centre-depolarising bipolar cells are hyperpolarised or depolarised, respectively, by passing hyperpolarising or depolarising currents through the horizontal cell membrane (Fig. 1*e*, 1 and 2).

Thus, in contrast to the conclusions of Piccolino and Gerschenfeld³, our data show that horizontal cell activity influences the membrane potential of bipolar cells in a way qualitatively consistent with the previously proposed role of horizontal cells in mediating surround effects. This result might be expected from published anatomical and physiological evidence—electron microscopy shows that horizontal cells are presynaptic to bipolar cells at presumed chemical synapses of conventional type^{1,6} while an indirect input to bipolar cells is provided by horizontal cell feedback onto cones⁷. Although the present results do not give information on the circuitry underlying the bipolar cell responses to horizontal cell stimulation, it seems reasonable to assume that both the direct and feedback pathways are involved. The participation of additional retinal elements in mediating the bipolar cell surround cannot be excluded.

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Bilayers containing gangliosides develop channels when exposed to cholera toxin

CHOLERA toxin, a protein of molecular weight 84,000, comprising five or six B and one A subunits, produces massive diarrhoea in man and other mammals by altering the transport of salt and water across the intestinal epithelium¹⁻⁴. These effects are mediated by a sequence of events leading to an increase in the concentration of cyclic AMP in the epithelial cells. The sequence involves binding of the toxin through its B subunits to specific receptors which seem to be the monosialoganglioside galactosyl-*N*-acetyl-galactosaminyl-(*N*-acetylneuraminyl)-galactosyl-glucosyl-ceramide (GM_1), and subsequent activation of adenyl cyclase⁵⁻⁷. We report here experiments which show that cholera toxin reacts with lipid bilayers containing GM_1 to form channels which allow ions present in the aqueous solution to traverse the membrane.

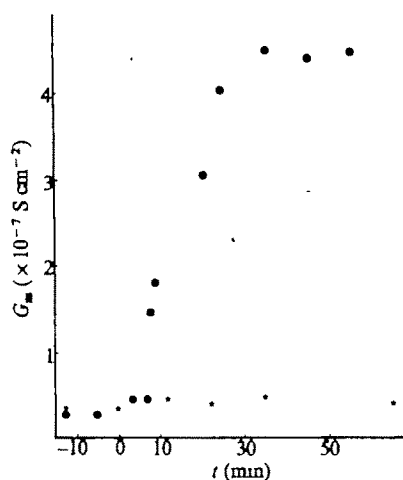
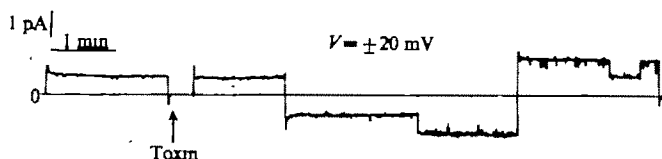


Fig. 1 Time course of development of membrane conductance (G_m) of a bilayer containing 3% G_{M1} in GMO (●) and one containing 3% G_{M2} in GMO (★) after addition at time $t = 0$ of 10 ng ml^{-1} (●) or 15 ng ml^{-1} (★) cholera toxin to one chamber. GMO concentration was 12 mg ml^{-1} .

Bilayers were formed from a mixture of glycerolmonooleate (GMO) (Nu-Chek) and G_{M1} or G_{M2} (*N*-acetylgalactosaminyl-*N*-acetylneuraminyl)galactosyl-glucosyl-ceramide) (Supelco) in the molar ratios indicated. The lipid mixture was dissolved in chloroform-methanol (2:1, v:v) and spread on the water-air interfaces of the bathing solutions. After allowing the solvents to evaporate (approximately 30 min), $2 \mu\text{l}$ of decane were added to each of the interfaces, a procedure which increased the stability of the membranes. The bilayers were then formed by the technique first described by Montal and Mueller⁸ and described in detail by Hall and Latorre⁹. The area of black film could not be determined accurately by visual means (the diameter of the holes used in these studies was 0.36 mm). Therefore, an estimate was made from the measured capacitance of the films (690 – 740 pF) and the value of the specific capacitance for this type of membrane formed in the absence of decane at the interfaces, by pretreating the hole with a 2% solution of squalene in pentane ($0.84 \pm 0.02 \mu\text{F cm}^{-2}$). The aqueous solutions present in the 1.5-ml chambers contained initially 100 mM NaCl buffered to $\text{pH } 7.0$ with $5 \text{ mM Na-phosphate}$ buffer. The electrical characteristics were determined using a pair of Ag-AgCl electrodes to inject and measure current, and a pair of calomel electrodes to measure zero-current membrane potentials. When appropriate, a portion of a freshly diluted solution of cholera toxin (Schwarz-Mann) was added to one of the chambers.

Figure 1 shows the results of experiments in which cholera toxin was added to the aqueous solution bathing one side of GMO bilayers containing either G_{M1} or G_{M2} . An abrupt and substantial increase in membrane conductance was observed in the former but not in the latter case. GMO membranes did not respond to choleragen addition (not shown). Apparently, a

Fig. 2 Recorder trace showing the development of membrane conductance and current fluctuations for a GMO membrane containing 1% G_{M1} . GMO concentration, 12 mg ml^{-1} . Cholera toxin was added to a final concentration of 3 ng ml^{-1} . The jump in current shown was the only one, although at later times, current fluctuations of smaller values were also observed.



specific interaction between G_{M1} and cholera toxin is necessary to produce an increase in membrane conductance. This point was substantiated by experiments in which choleragen was mixed with excess G_{M1} and then added to the aqueous solution bathing one side of a bilayer containing G_{M1} . No increase in membrane conductance was observed. Subsequent addition of cholera toxin not previously mixed with G_{M1} to the opposite side of the membrane produced the usual conductance increase.

Examination of records of membrane current at constant membrane voltage and higher time resolution reveals the presence of current fluctuations (Fig. 2). These records are consistent with a picture in which ion-conducting channels with

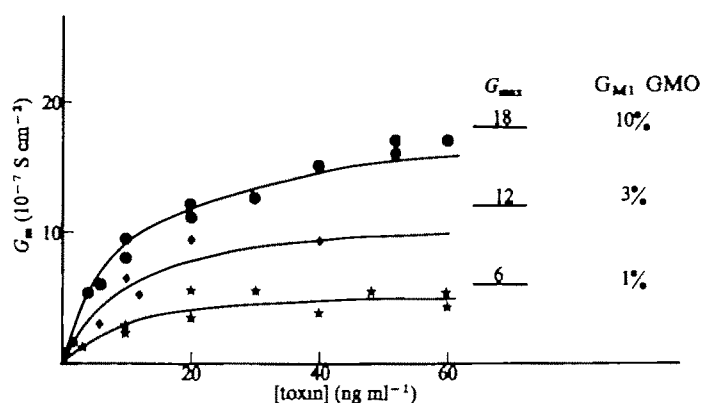


Fig. 3 Dependence of the zero-current, zero-voltage steady-state conductance (G_m) on the concentration of cholera toxin at different molar ratios of G_{M1} in GMO (12 mg ml^{-1}). The points for 1% and 10% were obtained in three different membranes, each of which was exposed to at least three different concentrations of toxin. Those for 3% are all from different membranes, averaged values. The lines were drawn according to the equation in the text, with $K_m = 1.2 \times 10^{-10} \text{ M}$ and G_{max} values as indicated

a conductance of the order of 20 – 40 pS cm^{-2} randomly open and close during the experiment. The ionic selectivity of the channels was evaluated by measuring the zero-current membrane potential in the presence of known differences in the concentration of Na, K and Cl in the two aqueous bathing solutions. The results indicated that the channels are twice as permeable to the monovalent cations as to chloride, with practically no selectivity for Na over K.

Figure 3 shows the dependence of membrane conductance on cholera toxin concentration at three different mol fractions of G_{M1} in the membrane-forming solution. The bilayer conductance levelled off as the toxin concentration in the aqueous phases was increased, and so the data were fitted using a saturating type of relation between membrane conductance and toxin concentration:

$$G_m = \frac{G_{max}[\text{toxin}]}{K_{1/2} + [\text{toxin}]}$$

where G_m is the steady-state membrane conductance, G_{max} is the maximum membrane conductance at high toxin concentration, [toxin] is the concentration of cholera toxin in the aqueous phase and $K_{1/2}$ is the concentration of toxin which produced half the maximum conductance increase. The value of $K_{1/2}$ required to fit the data at the three concentrations of G_{M1} used is $1.2 \times 10^{-10} \text{ M}$, similar to the value found by Cuatrecasas for the interaction of choleragen with fat cell membranes⁵.

These data support a picture in which cholera toxin reacts with the sugar residues in the G_{M1} molecules in the membrane surface to produce a complex that forms ion-conducting channels. We are now investigating the size and kinetic properties of the single channels and the role of the different subunits of cholera toxin in

the process of channel formation. These experiments may help to clarify the mechanism by which cholera toxin, and perhaps other agonists, activate adenyl cyclase.

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Human urinary kallikrein converts inactive to active renin and is a possible physiological activator of renin

WE have found that human urinary kallikrein converts inactive plasma renin *in vitro* to an enzyme with renin-like activity. This observation is interesting because renal kallikrein, the presumed precursor of urinary kallikrein¹, is thought to be situated in or on cells of the distal convoluted tubule, including the macula densa cells¹⁻³, which are adjacent to the storage site of renin, the juxtaglomerular cells⁴. Also, human urinary kallikrein is a trypsin-like enzyme of highly restricted specificity⁵, and although trypsin is capable of activating inactive renin⁶, we have found that kallikrein is at least 10 times more active. Therefore it seems possible that urinary kallikrein is the *in vivo* converting enzyme of prorenin and may activate the renin-angiotensin-aldosterone hormonal system, as it participates in sodium and potassium homeostasis⁴ and in the blood pressure maintenance of a majority of hypertensive patients⁷.

Inactive plasma renin can be converted *in vitro* to active renin by trypsin⁶, by exposure to cold (cryoactivation)^{8,9}, or by dialysis to pH 3.3 with subsequent restoration of neutral pH (ref. 10). We have shown that in plasma the major portion of the increase in renin following dialysis to pH 3.3 occurs at alkaline pH (refs 11, 12). Like cryoactivation, the alkaline phase of 'acid' activation seems to involve an unidentified endogenous neutral serine protease^{11,12}. As acidification of plasma destroys endogenous protease inhibitors^{13,14}, and as activation of inactive renin by endogenous proteases following acidification proceeds very slowly, we were able to use acidified plasma to test the potency of small amounts of highly purified urinary kallikrein¹⁵ on the conversion of inactive to active renin.

Pooled human plasma (50 ml, in presence of EDTA) with renin activity¹⁶ of 1.5 ng angiotensin I ml⁻¹ h⁻¹ was dialysed to pH 3.3 for 24 h at 4 °C. Two 1-ml samples were further dialysed from pH 3.3 to 7.4 at 4 °C for 24 h after which renin averaged 8.5 ng ml⁻¹ h⁻¹ (Fig. 1). Plasma renin activity was 4.5 ng ml⁻¹ h⁻¹ in the remainder of the pool which was adjusted to pH 7.4 and there was no further increase during 1 h incubation at 25 °C and pH 7.4. When urinary kallikrein or trypsin was added to this pool for 1 h at 25 °C, a 66% increase in renin activity was observed with urinary kallikrein 2 × 10⁻⁷ M but only a 39% increase with trypsin 2 × 10⁻⁶ M (Fig. 1). Maximum activation (that is, to 8.3 ng ml⁻¹ h⁻¹) was achieved by urinary kallikrein 4 × 10⁻⁷ M, whereas trypsin 8 × 10⁻⁶ M did not quite achieve maximum activation.

In another study urinary kallikrein at 0, 2.0 and 3.1 × 10⁻⁶ M was added to untreated plasma and incubated for 2 d at -4 °C. In the absence of urinary kallikrein there was a small increase in renin activity due to cryoactivation⁹. However, urinary kallikrein caused a concentration-dependent increase in the rate of cryoactivation (Fig. 2), thus demonstrating that activation of inactive renin by kallikrein does not depend on previous acidification.

Although inactive renin is present in the plasma of most anephric patients¹⁷, the data in Fig. 3 demonstrate that it is also of renal origin. Active and inactive plasma renin were measured in simultaneously collected renal vein and inferior vena caval (IVC) samples from a patient with renal artery stenosis. Active and inactive renin levels were the same in the IVC and right renal vein, but there were marked increments in both forms of renin in renal venous plasma from the left side, thus demonstrating renal secretion of both active and inactive renin.

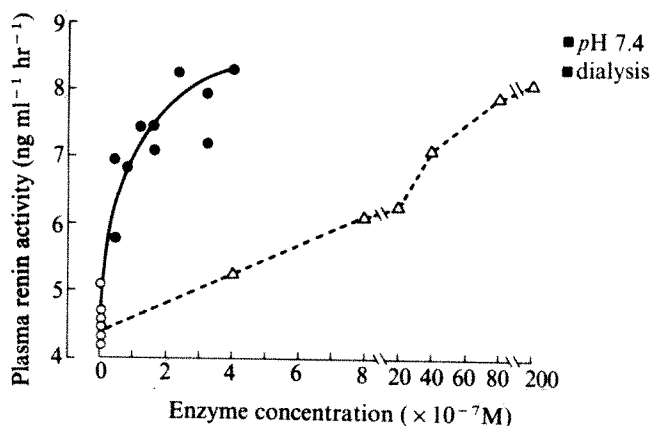


Fig. 1 Activation of inactive renin in previously acidified plasma by urinary kallikrein, trypsin or endogenous serine proteases. Pooled human plasma (50 ml; EDTA) was dialysed to pH 3.3 for 24 h at 4 °C (0.05 M glycine-HCl, 0.1 M NaCl, 3 mM Na₂EDTA). This destroys endogenous protease inhibitors^{13,14} and activates about one-third of inactive plasma renin¹¹. Three methods of activation of the remaining inactive renin were tested. ■, Two 1-ml samples were further dialysed to pH 7.4 for 24 h at 4 °C (0.10 M sodium phosphate, 0.05 M NaCl, 3 mM Na₂EDTA) during which time endogenous proteases slowly activated inactive renin. The remainder (48 ml) was adjusted to pH 7.4 at 0 °C with 5 ml 4 M Tris, pH 7.4, and 0.5 ml 2 M NaOH. ●, Pure urinary kallikrein¹⁵ (specific activity 1094 µg equivalents bradykinin min⁻¹ mg⁻¹ at pH 8.5 and 37 °C); or △, trypsin (Boehringer Mannheim, specific activity 33 U mg⁻¹); or ○, distilled water, were added to 0.5 ml samples which were then incubated at 25 °C for 1 h. Activation was stopped by adjustment to pH 5.7 and addition of phenylmethylsulphonyl fluoride (PMSF) 1.5 mmol l⁻¹. Renin activity was then measured by incubation at pH 5.7 and 37 °C (ref. 16) after addition of partially purified human renin substrate¹¹. Results for urinary kallikrein were from three separate experiments.

Stop-flow² and histological studies³ have suggested that renal kallikrein is located in the cells lining the distal convoluted tubule extending from the juxtaglomerular apparatus (where renin is synthesised) to the collecting duct. Its activity has been associated with the plasma membrane and/or endoplasmic reticulum^{1,18} of cells from the renal cortex, although lysosomal fractions have also been implicated¹⁹. Thus, kallikrein may be present in the renin-secreting cells of the juxtaglomerular apparatus. Urinary kallikrein is reported to have a more restricted specificity than trypsin⁵ and is often catalytically less efficient^{20,21}. Its greater potency for activating inactive renin would therefore seem to favour specific interaction. This observation, together with its location in the kidney, suggests that kallikrein might be a prorenin converting enzyme. However, other mechanisms may be involved, as inhibitors of sulphhydryl-dependent enzymes have been reported to block conversion of

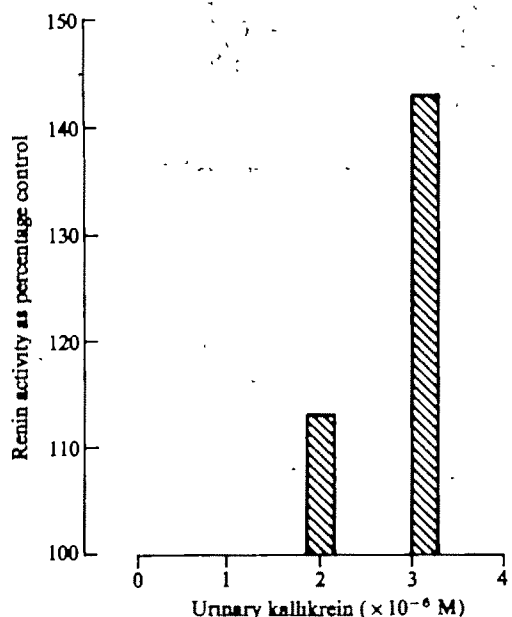


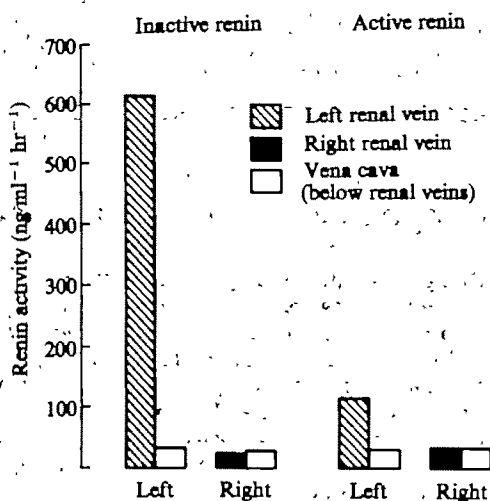
Fig. 2 Potentiating effect of urinary kallikrein on the rate of cryoactivation of inactive plasma renin: 100 or 200 μ l (2×10^{-6} and 3.1×10^{-6} M) purified urinary kallikrein or distilled water was added to 0.3 ml plasma (EDTA) which was then incubated at -4°C for 38 h (96 h are required for cryoactivation to reach a plateau). Renin was then assayed at pH 5.7 and 37°C in the presence of 1.5 mM PMSF. Renin activity, measured after cryoactivation in the presence of urinary kallikrein, is expressed as a percentage of that measured after 38 h cryoactivation alone.

large to small molecular weight hog renal renin with a resultant small change in activity²².

If kallikrein activates inactive renin *in vivo*, this would be the second link between the kallikrein and renin systems, as angiotensin-converting enzyme converts angiotensin I to angiotensin II and also hydrolyses bradykinin to inactive products²³.

So far, urinary kallikrein is the most potent activator of inactive renin yet described and may be strategically located to

Fig. 3 Unilateral renal secretion of active and inactive renin from a hypertensive patient with left renal artery stenosis. Blood specimens were collected from the IVC simultaneously with each renal vein blood collection. Inactive plasma renin was measured as the difference between endogenous renin activity and the renin activity following activation by trypsin (1 mg per ml plasma at -4°C for 1 h). There was no difference in active or inactive renin between the IVC and right renal vein, whereas a marked increment in both renins was observed from the left side, thus demonstrating the renal origin of both active and inactive renin.



participate in renin biosynthesis. Whether the enzyme is part of a biological control system for the release of renin and for the accomplishment of sodium, potassium and blood pressure homeostasis, remains to be established.

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Specific and irreversible block of the myotropic action of angiotensin II

COMPETITIVE inhibitors of the octapeptide hormone angiotensin II (AT), Asp-Arg-Val-Tyr-Val-His-Pro-Phe, have been synthesized by several groups¹. These inhibitors, like the hormone, are quickly degraded, and no irreversible or even long-acting specific inhibitors have been available. Paiva *et al.*² have attempted to produce such inhibitors using affinity labelling analogues, but with poor results. We report here a specific and irreversible inhibition of the response of rabbit aorta strips to AT by a photolabile analogue of AT.

We previously described the synthesis³ and the biological activities⁴ of the AT analogue Sar-Arg-Val-Tyr-His-Pro-(4'-azido)Phe (=N₃-AT) in the absence of ultraviolet radiation. Also, (4'-azido)Phe has been successfully used as a photoaffinity label in studies on chymotrypsin⁵ and ribosomes⁶. In the present study, rabbit aorta strips were suspended in Krebs' solution according to the method of Rioux *et al.*⁷. Photolysis was carried out with four parabolic mercury vapour lamps (Westinghouse JC PAR-38) equipped with black light filters surrounding a tissue bath. The light was further filtered through pyrex glass and

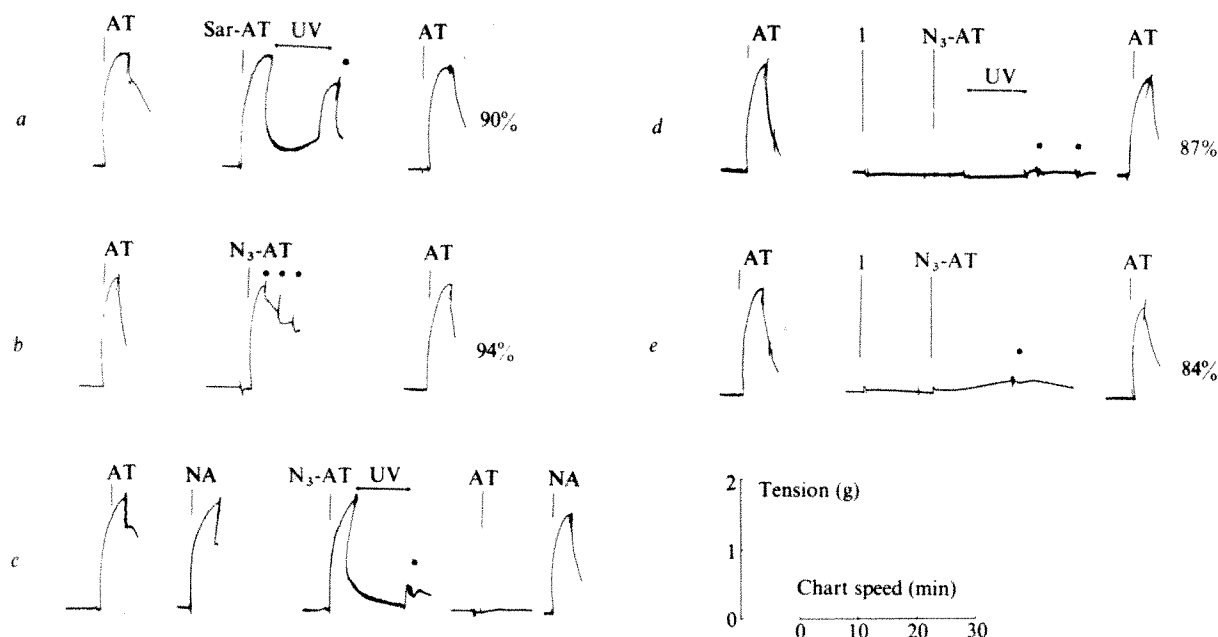


Fig. 1 Contraction of rabbit aorta strips in various conditions. The interval between injections of angiotensin II (AT), 200 ng ml⁻¹, (Sar¹)-angiotensin II (Sar-AT), 200 ng ml⁻¹, or its photolabelling analogue (Sar¹(4'-azido)Phe⁸)-angiotensin II (N₃-AT), 200 ng ml⁻¹, was 90 min. Noradrenaline (NA), 50 ng ml⁻¹, was injected 30 min after AT, and (Leu⁸)-angiotensin II (I), 5,000 ng ml⁻¹, was added 15 min before the agonist. All tests have been carried out under total exclusion of UV radiation except when indicated. Dots indicate washing of the tissues. Contractions of the rabbit aorta strips suspended in tissue baths were recorded on a Grass polygraph with isometric force transducers. All other experimental details are as described by Rioux *et al.*⁷.

water so that almost only the 365-nm band of the mercury spectrum reached the tissue.

When tissues contracted by (Sar¹)-AT were irradiated, they relaxed immediately (Fig. 1a), analogous to the results reported by Furchgott *et al.*⁸. If the irradiation is discontinued the tissues regain their initial degree of contraction and are normally responsive to further applications of stimulants. In the dark, N₃-AT acts as a reversible agonist of the AT receptor (Fig. 1b); it has the same intrinsic activity as (Sar¹)-AT and a relative affinity of 31%. If the tissues contracted by N₃-AT (Fig. 1c) are irradiated, the strips relax as they do under the influence of light, but they do not recontract when the light is removed. They also lose their responsiveness to AT but not to noradrenaline (Fig. 1c), histamine, bradykinin, 5-hydroxytryptamine or potassium (not shown), agents acting on receptors different from those of AT. Further applications of AT during the following 2–6 h do not induce any significant rise in tension. If the same experiment is carried out in the presence of the competitive inhibitor (Leu⁸)-AT at saturating concentrations, the tissues recover their responsiveness to AT (Fig. 1d). The slight decrease in response observed is due to some desensitisation by the inhibitor and it also occurs in the same experiment without irradiation (Fig. 1e).

In some preparations (one out of five), in which the desensitisation of the tissues to AT achieved with photolysis of N₃-AT was less than 80%, the treatment was repeated and resulted in an almost complete inactivation.

Two possible reactions of the tissue were expected with a specific covalent block by an agonist. (1) The tissue stays contracted due to a continuous stimulation of the response by a stable hormone–receptor complex. This phenomenon has been reported with gastrin⁹. (2) Covalent attachment of the hormone to the receptor induces a permanent loss of hormone response, because stimulation occurs only when the receptor changes from 'bound' to 'unbound' or the reverse. If the hormone is irreversibly attached this dynamic process cannot occur again. We therefore conclude that, under photolysis, N₃-AT covalently and specifically blocks the receptor for AT. Studies using radioactive analogues of N₃-AT are now in progress in an attempt to isolate the AT receptor from arterial smooth muscle.

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Evidence for an essential DNA component in the Scrapie agent

ONE of the enigmas of microbiology has been the nature of the aetiological agent causing scrapie disease in sheep and goats. The unusual resistance of infectivity to heat, formaldehyde, nucleases and UV irradiation has stimulated many hypotheses, including speculation that the scrapie agent may lack nucleic acid¹. This stability to physical and chemical inactivation has been due in part to the intimate association of the agent with the cell membrane^{2,3}, making it impossible to obtain soluble fractions of brain tissue containing high titres of infectivity. A

hamster model of scrapie disease has been developed^{4,5} that has shorter incubation periods and higher brain titres of infectivity than any other animal model. Studies on the subcellular distribution of scrapie agent in hamster brain have shown that 8–9% of the total infectivity can be found in membrane-free high-speed supernatant (HSS) fractions⁶. Infectivity in the HSS exists as small heterogeneous complexes which are resistant to detectable enzymatic degradation, but are more sensitive to inactivation by heat and UV irradiation than are cruder brain preparations⁷. We report here further partitioning of scrapie infectivity in the HSS using two methods of separation, hydroxyapatite chromatography and polyacrylamide gel electrophoresis, both of which result in isolation of a fraction of scrapie infectivity that is inactivated by deoxyribonuclease (DNase). We therefore conclude that the scrapie agent contains an essential DNA component.

Hydroxyapatite chromatography was performed as reported previously⁷. Briefly, brain homogenates in 12 mM sodium phosphate (pH 7.2) are centrifuged at 100,000g for 18 h in a Beckman 42.1 rotor and the supernatant fractions applied to a hydroxyapatite column at room temperature. Scrapie infectivity eluted in the 0.48 M sodium phosphate wash, and representing 1% of the total input infectivity to the column, was diluted 1:4 with buffer (100 mM Tris, 0.15 M NaCl, 12 mM sodium phosphate, pH 7.2), then treated with DNase I (Worthington), RNase A (Worthington) or proteinase K (Boehringer-Mannheim) at concentrations of 100 $\mu\text{g ml}^{-1}$, 50 $\mu\text{g ml}^{-1}$ or 62 $\mu\text{g ml}^{-1}$, respectively. Magnesium chloride (20 mM) was added to the tube containing DNase and also to the control tube which received no enzyme treatment. All preparations were then incubated in a water bath at 37 °C for 60 min. To ensure that the enzymes used were biochemically active in the conditions stated, they were individually tested on radioisotope-labelled substrates which were added directly to the scrapie sample to be treated. Scrapie infectivity was measured by intracerebral inoculation of weanling hamsters and endpoints calculated 16 weeks after injection using the method of Spearman and Kärber⁸. As can be seen in Table 1, only treatment with DNase resulted in substantial reduction of infectivity.

Because the above experiment indicated that infectivity was resistant to proteolytic digestion, a second sample of HSS was digested with 100 $\mu\text{g ml}^{-1}$ proteinase K before adsorption to hydroxyapatite. The hydroxyapatite eluate from the proteinase K-digested HSS contained a 10-fold higher titre than that of the undigested HSS, and a greater proportion of the total scrapie infectivity was inactivated with DNase (Table 1).

In support of the above findings, we have since succeeded in obtaining a purified preparation of scrapie agent of higher titre using ammonium sulphate precipitation of the HSS and separation of the resuspended precipitate by polyacrylamide gel electrophoresis⁹. Enzyme treatment of scrapie infectivity from these gels (Table 1), in conditions similar to those described for the treatment of the hydroxyapatite eluates, also resulted in a significant decrease in scrapie activity after exposure to DNase (100 $\mu\text{g ml}^{-1}$), but not to RNase A (100 $\mu\text{g ml}^{-1}$) or proteinase K (100 $\mu\text{g ml}^{-1}$).

These results show that as the heterogeneous infectious complexes in the HSS are separated, either by hydroxyapatite

chromatography or polyacrylamide gel electrophoresis, fractions of infectivity can be detected which are sensitive to treatment with DNase. It is, therefore, reasonable to conclude that the scrapie agent contains an essential DNA component. This finding is compatible with other studies^{3,10,11} which have detected low-molecular-weight DNA sequences in scrapie-infected brain, but which have been of questionable significance because of the inability to relate these nucleic acids to infectivity. However, the exact nature, size and possible association of this scrapie-specific DNA with other macromolecules requires further study.

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Highly conserved DNA of Ti plasmids overlaps T-DNA maintained in plant tumours

LARGE tumour-inducing (Ti) plasmids in *Agrobacterium tumefaciens*¹ are responsible for crown gall disease in dicotyledonous plants^{2,3}. Plant tumour cells cultivated *in vitro* in the absence of the inciting bacterium maintain multiple copies of part of the Ti plasmid^{4,5}. The experiments presented here show that part of the Ti plasmid maintained in tumour cells (T-DNA) is highly conserved among diverse Ti plasmids.

Ti plasmids from a variety of natural isolates of *A. tumefaciens* exhibit only partial DNA homology^{6,7}. Their fingerprints, produced by cleavage of plasmid DNA with restriction endonucleases, are markedly different^{8–11}. Ti-plasmid-borne genetic traits (octopine and nopaline metabolism)^{2,3,12–15} suggest that Ti plasmids are of three types, and physical studies confirm this^{6–11}. Schell and his collaborators¹⁶ have suggested that since common tumour-inducing functions are a feature of every Ti plasmid, a region of homologous DNA should be detectable in all types. We have mapped the plasmid pTi-B6-806 (ref. 17) and located the T-DNA, the region of this plasmid that is maintained in E9 tobacco tumour (Fig. 1)^{4,18}. We report

Table 1 Enzyme treatment of scrapie infectivity separated by hydroxyapatite chromatography (HA) or polyacrylamide gel electrophoresis (PAGE)

Enzyme treatment	HA (undigested)	HA (predigested)	PAGE
None	4.0*	5.0	7.5
RNase A (50–100 $\mu\text{g ml}^{-1}$)	4.2	5.0	7.8
Proteinase K (62–100 $\mu\text{g ml}^{-1}$)	4.0	ND†	7.5
DNase I (100 $\mu\text{g ml}^{-1}$)	2.5	2.8	4.2

*log₁₀ LD₅₀ ml⁻¹.

†Not determined.

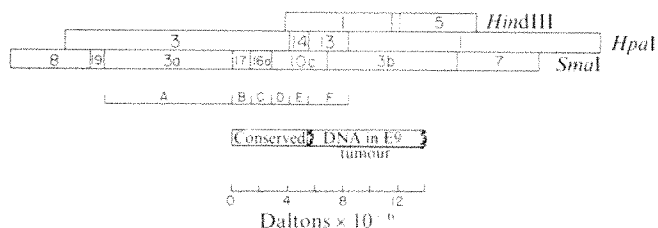


Fig. 1 Map of *Sma*I, *Hpa*I and *Hind*III restriction endonuclease cleavage sites in part of pTi-B6-806. Evidence for the map order is given in detail elsewhere^{17,18}. Certain fragments of pTi-B6-806 used in this study were designated A-F as indicated above for clarity. Evidence for the boundaries of the DNA detected in E9 tumour (T-DNA) is presented elsewhere^{4,5,18}.

here DNA hybridisation studies that test this region of pTi-B6-806 for homology with six representative Ti plasmids.

A map of the sector of pTi-B6-806 that is relevant to this study is presented in Fig. 1. For the DNA hybridisation studies described below, pTi-B6-806 DNA fragments to be used as probes were isolated after preparative agarose gel electrophoresis of plasmid DNA digests⁴. Fragments were labelled by the 'nick translation' reaction¹⁹.

Figure 2 presents *Sma*I cleavage patterns of the Ti plasmids examined here. Nopaline-type plasmids are diverse, and are

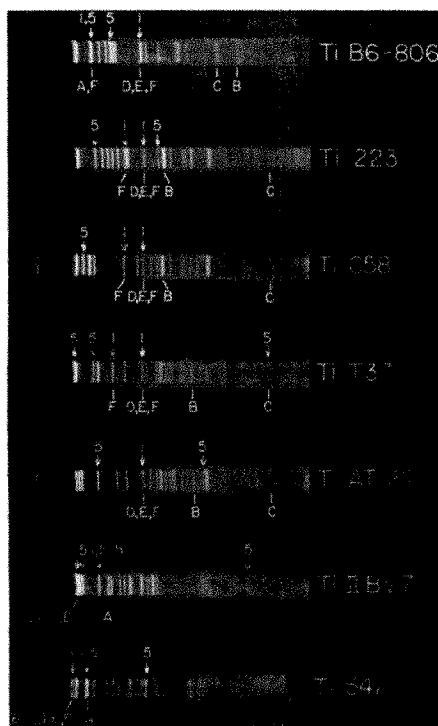


Fig. 2 *Sma*I cleavage patterns of diverse Ti plasmids. Descriptions and sources of bacterial strains are detailed elsewhere¹⁰. Plasmid DNA (2.5 µg) isolated as described previously¹⁰ was adjusted to 0.02 M Tris, pH 7.5, 15 mM KCl, 6 mM MgCl₂ and treated with sufficient *Sma*I to give a complete digest in 2 h at 30 °C. *Sma*I was purified by the procedure of Lyle Brown, Oregon State University (private communication). Digests were subjected to horizontal slab gel electrophoresis¹⁰ and bands were visualised by staining for 20 min with ethidium bromide (10 µg/ml) and illuminating on a Blak-Ray Transilluminator (UV Products Inc.). Arrows above each digest indicate the positions of fragments that hybridised strongly (solid arrows) or weakly (dotted arrows) with *Hind*III fragments 1 and 5; the probe is indicated by the number above each arrow. The letters beneath each digest indicate the positions of fragments that hybridised with probes A-F, as defined in Fig. 1.

represented by pTi-223, pTi-C58, pTi-T37, pTi-AT181 and pTi-IIBV7. The only known null-type plasmid is pTi-542⁹. Octopine-type plasmids are highly conserved⁶⁻¹⁰ and are represented by pTi-B6-806, the homologous DNA control. On the basis of DNA homology measurements^{6,7} pTi-IIBV7 and pTi-542 are more closely related to the octopine-type plasmid than are any of the other plasmids studied here.

Digest fragments of Ti plasmids (Fig. 2) were transferred from the agarose gel to sheets of cellulose nitrate (Schleicher and Schuell, B6 membrane) by a modification²⁰ of the Southern blotting procedure²¹. Transfers were cut into longitudinal strips for hybridisation with various labelled DNA fragments.

*Hind*III fragment 1 labelled DNA hybridised with most of the heterologous plasmids extensively, as shown in the autoradiograms of Fig. 3 (data not shown for pTi-542). In four of the heterologous plasmid digests, the labelled DNA bound almost exclusively to a fragment of approximately the same electrophoretic mobility as *Sma*I fragment 10c of pTi-B6-806

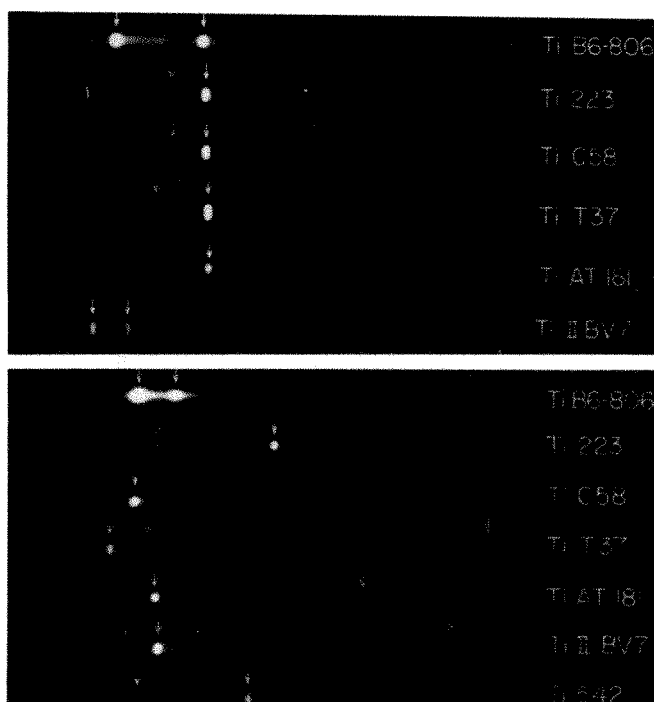


Fig. 3 Autoradiograms of hybrids formed with labelled *Hind*III fragments 1 and 5 as probes. DNA fragments used as probes were labelled to specific activities of $1-50 \times 10^6$ c.p.m. per µg by incorporation of [α -³²P]thymidine triphosphate (90-150 Ci mmol⁻¹) or [α -³²P]deoxycytidine triphosphate (1,500 Ci mmol⁻¹) (NEN) using the nick translation reaction¹⁹. Cellulose nitrate transfer strips prepared from the gel of Fig. 2 or similar gels were allowed to hybridise with labelled DNA fragments (10⁵ c.p.m. per ml in 2.5 × SSC at 63 °C for 18 h) as described elsewhere (SSC is 0.15 M NaCl, 0.015 M trisodium citrate). The solid and dotted arrows above the bands indicate the intensity of hybridisation of the probes, and correspond to solid and dotted arrows in Fig. 2. Upper set of autoradiograms, *Hind*III fragment 1 probe. Lower set, *Hind*III fragment 5 probe.

(solid arrows marked 1 in Fig. 2). In the remaining two cases, pTi-IIBV7 and pTi-542, two fragments of higher molecular weight bound the labelled probe less efficiently. As expected from the map position of *Hind*III fragment 1 (Fig. 1), this probe hybridised strongly with pTi-B6-806 *Sma*I digest bands 3 and 10.

Thermal dissociation profiles of the DNA/DNA duplexes formed with *Hind*III fragment 1 probe are presented in Fig. 4. The *T*_m (midpoint of thermal dissociation) of homologous duplexes, 86 °C, is 3-5 °C higher than that of the heterodu-

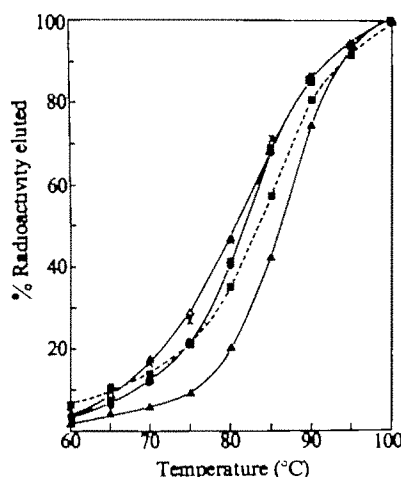


Fig. 4 Thermal dissociation profiles of *Hind*III fragment 1 duplexes. The autoradiogram of the upper portion of Fig. 3 was used as a guide to locate bands of DNA duplexes corresponding to the solid arrows. The cellulose nitrate segment bearing these duplexes was excised and placed in a 10×75 mm test tube. The segment was eluted with 1 ml aliquots of 1×SSC at 22°, 60°, 65 °C and so on and radioactivity eluted at each temperature was assayed. Radioactivity eluted at 22 °C and that remaining on the cellulose nitrate segment at the end of the melt were negligible. Total radioactivity eluted was 1,500–2,000 c.p.m. in all cases. ▲, pTi-B6-806 ($T_m = 86^\circ\text{C}$); △, pTi-IIBV7 ($T_m = 81^\circ\text{C}$); □, pTi-223 ($T_m = 82^\circ\text{C}$); ●, pTi-CS8 ($T_m = 82^\circ\text{C}$); ○, pTi-T37 ($T_m = 82^\circ\text{C}$); ×, pTi-AT181 ($T_m = 82^\circ\text{C}$), ■, pTi-542 ($T_m = 83.5^\circ\text{C}$)

plexes, indicative of 3–5% base-pair-mismatching in the latter²⁴.

Labelled *Hind*III fragment 5 of pTi-B6-806 hybridised less extensively to all heterologous plasmids than to the homologous one (Fig. 3). The arrows marked 5 in Fig. 2 indicate the fragments that bound this labelled probe well (solid arrows) or poorly (dotted arrows). We have not examined in detail which part of *Hind*III fragment 5 is conserved in the heterologous Ti plasmids.

We obtained a more detailed picture of the extent of the conserved DNA region within and to the left of *Hind*III fragment 1 by using as labelled probes *Sma*I and *Hpa*I digest fragments of pTi-B6-806. The 42 autoradiograms from these experiments are not shown here, but the results are summarised in Fig. 2. The labelled probes are designated by serial letters A–F, corresponding to their left-to-right map order as indicated in Fig. 1. Probe A hybridised with only two heterologous plasmids, pTi-542 and pTi-IIBV7, at positions marked A in Fig. 2. In contrast to probe A, probe F hybridised efficiently with most of the Ti plasmids, but very poorly with pTi-542 and not detectably with pTi-IIBV7 (Fig. 2).

Probes B, C, D and E hybridised with all of the Ti plasmids examined, and must therefore constitute a region of DNA common to all (Fig. 2). This common DNA region extends on the map from *Sma*I fragment 17 to *Hpa*I fragment 14, a region of 5.5×10^6 daltons or less. Probe F (*Hpa*I fragment 13) could also be considered a part of the common DNA region, for its DNA sequences are preserved on most of the Ti plasmids examined. However, the region of conserved DNA indicated in Fig. 1 does not include this imperfectly conserved fragment. The T-DNA portion of pTi-B6-806 (Fig. 1) is at least contiguous with this conserved DNA element, and since the left boundary of T-DNA is not precisely known, it is possible that it overlaps the conserved DNA region. The T-DNA of a T37 tumour line under investigation in this laboratory contains all of this conserved DNA region from pTi-T37 (ref. 22).

If there have been no gross rearrangements of DNA in these plasmids during evolution, the results presented here predict

the serial map order of the heterologous Ti plasmid fragments to be the same as that of the pTi-B6-806 probe fragments A through F, to which they hybridise (Fig. 2). We have confirmed this order by conventional mapping techniques¹⁷ for pTi-T37 (ref. 23).

It seems reasonable to predict that crown gall tumour lines incited by bacterial strains carrying the heterologous Ti plasmids studied here will contain segments of those plasmids that are proximal to the conserved DNA sequences. The results presented here show, however, that a significant portion of the T-DNA of pTi-B6-806 comprises DNA sequences not common to all Ti plasmids. The right half of *Hind*III fragment 1 is not conserved, for all of the hybridisation of this probe is ascribable to probes E and F (Figs 1 and 2), which overlap its left end. The non-conserved part of the T-DNA may code for octopine production in pTi-B6-806, a trait not shared by nopaline-type Ti plasmids, which code for nopaline production by tumours^{1,9,13,14}.

The genetic evidence of Depicker *et al.*²⁵ in the accompanying paper indicates that the common DNA sequences analysed here do indeed code for functions essential to oncogenicity. This DNA could code for functions related to the process of transformation, or for functions required in the tumour cell for maintenance of the transformed state. Transcripts of Ti plasmid DNA have been detected in crown gall tumour tissue²⁶, but it is not clear whether the common DNA region of pTi-B6-806 is transcribed. Further evidence is needed for an understanding of the role of this highly conserved DNA element in crown gall tumour induction.

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Homologous DNA sequences in different Ti-plasmids are essential for oncogenicity

LARGE plasmids, called Ti-plasmids¹, are responsible for the transformation of dicotyledonous plants by *Agrobacterium tumefaciens*²⁻⁵. A comparison of Ti-plasmids derived from different *Agrobacterium* strains⁶⁻¹⁰ revealed two major groups of homology. These correlate with the Ti-plasmid-determined properties of octopine or nopaline utilisation by Ti-plasmid-harboring agrobacteria^{2,5,11-13}, and octopine or nopaline synthesis by Ti-plasmid-transformed plant cells^{11,13}. It was originally suggested that octopine or nopaline synthesis in crown-gall tissues might be mediated by bacterial genes, transferred to the transformed plant cells¹⁴; this can now be restated as being mediated by Ti-plasmid genes, and is supported both by genetic data¹⁵ and by hybridisation studies¹⁶. The capacity to determine both the catabolism and the synthesis of one or the other of these or similar compounds seems to be the dominant evolutionary significance of the Ti-plasmids and one can therefore speak of 'octopine'- and 'nopaline'- Ti-plasmids. In view of the fact that the different types of Ti-plasmids only have a limited degree of DNA sequence homology⁶, but produce transformed plant cells with similar phenotypes, it was conceivable that at least part of the common DNA sequences would be directly and/or indirectly involved in oncogenicity. We report here the finding of a specific DNA segment which is highly conserved among several different *Agrobacterium tumefaciens* Ti-plasmids. We also give genetic evidence that this region is essential for oncogenicity and overlaps with the Ti-plasmid DNA found in transformed plant cells.

The Ti-plasmid of strain ACH5 was used as an example of an octopine Ti-plasmid. This strain contains only one large plasmid (molecular weight 120×10^6), which was shown by heteroduplex analysis⁸ to be completely homologous to other octopine Ti-plasmids, such as the Ti-plasmid of strain B6S3. pTi-ACH5 DNA, isolated as described previously¹³ and radioactively labelled *in vitro* by nick translation¹⁸, was hybridised with the unlabelled DNA fragments obtained by digestion of the pTi-DNA from the nopaline strains C58 and K14 with various restriction endonucleases. Strong hybridisation was observed with only one segment of both nopaline Ti-plasmids. This segment was contained in one particular fragment of an *EcoRI* digest (fragment 1, MW 9.5×10^6), as can be seen for pTi-C58 DNA in Fig. 1. The largest band of the pTi-C58 *EcoRI* restriction pattern is, in fact, a doublet. After separation of the two fragments, it was demonstrated that the dominant hybridisation with pTi-ACH5 DNA was located in the *EcoRI*-1 fragment of pTi-C58 DNA. Other nopaline Ti-plasmids (from strains 223, T37, 396) were similarly tested for homology with pTi-ACH5 and it was found that they all contained an *EcoRI* fragment of MW 9.5×10^6 that showed a strong hybridisation with pTi-ACH5. This was particularly striking, as Ti-plasmids in avirulent mutant strains derived from strain K14 (ref. 15) did not exhibit this strong hybridisation.

Reciprocal experiments were carried out by hybridising total labelled pTi-C58 DNA with unlabelled DNA fragments of the octopine plasmid pTi-ACH5. Strong hybridisation was observed with the pTi-ACH5 *HindIII* fragment 1 (8.0×10^6 daltons) as can be seen in Fig. 1. These results thus indicated that this region is common to octopine and nopaline Ti-plasmids. Note here that various other fragments of these two types of Ti-plasmids exhibited a variable degree of hybridisation, as can be seen in Fig. 1, probably indicating a lesser extent of homology rather than an experimental artefact, as it was found that the extent of these hybridisations depended on the stringency of the hybridisation conditions used. Thus, it was found that in standard hybridisation conditions (see legend to Fig. 1), up to 80% of all the fragments of the pTi-ACH5 plasmid

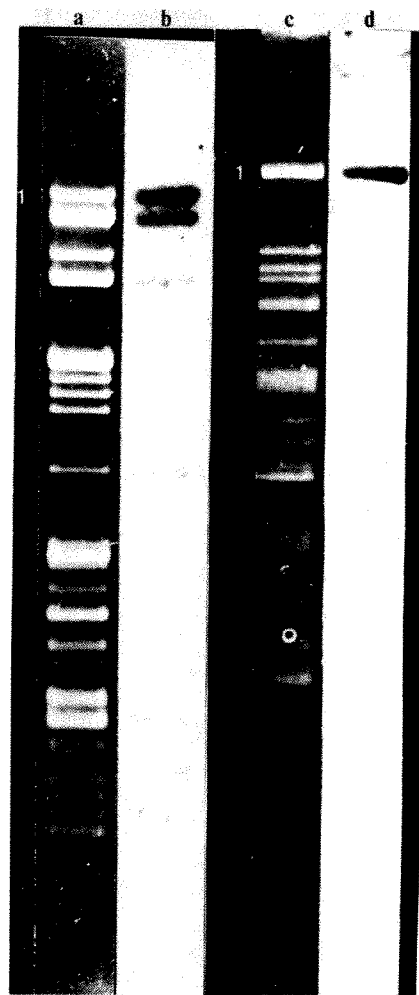
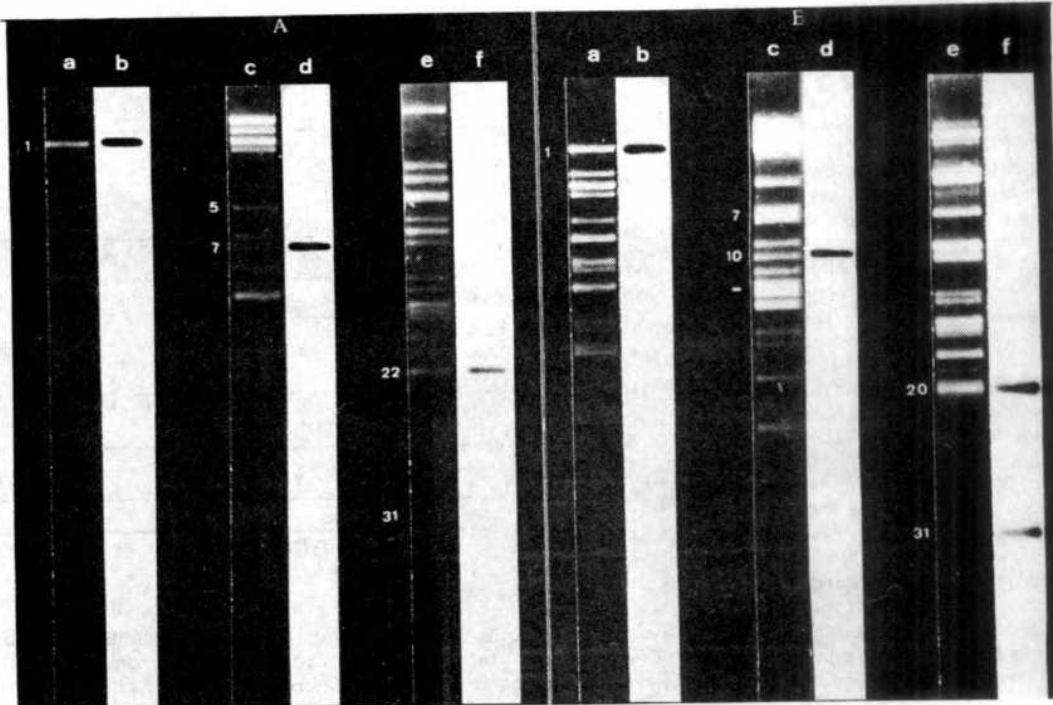


Fig. 1 Homology between octopine and nopaline Ti-plasmids. Plasmid DNA was prepared from wild-type strains ACH5 and C58 (natural isolates), as described previously¹³. The restriction enzymes *EcoRI* and *SmaI* were purified in this laboratory. The conditions for digestion were 90 mM Tris, pH 7.5, 50 mM NaCl and 5 mM MgCl₂ for *EcoRI*, 15 mM Tris, pH 8.5, 15 mM KCl and 6 mM MgCl₂ for *SmaI* and 7 mM Tris, pH 7.5, 60 mM NaCl and 7 mM MgCl₂ for *HindIII*. The digests were separated in 0.8% agarose, formed in 40 mM Tris-acetic acid, pH 8, 20 mM NaAc, 1 mM EDTA and 0.5 µg ml⁻¹ ethidium bromide, on a horizontal slab gel apparatus²⁰. Fragments of decreasing molecular weight were represented by increasing numbers; only one number was given to single bands consisting of several fragments. The λ-*EcoRI* and λ-*HindIII* fragments were used as molecular weight standards. After electrophoresis (40 V, 16 h) and photographing of the restriction pattern²¹, the DNA fragments were transferred to a nitrocellulose filter (Schleicher and Schuell no. B6) according to the Southern blotting procedure¹⁷. By nick translation, the probe DNA was labelled with ³²P to specific activities of 1–30 × 10⁶ c.p.m. µg⁻¹, using [α-³²P]-guanosine triphosphate (Amersham, 350 Ci mmol⁻¹)¹⁸. After preincubation of the filters for 3 h at 75 °C in 2 SSC, containing 0.02% polyvinylpyrrolidone, 0.02% Ficoll, 0.02% bovine serum albumin (BSA) and 20 µg ml⁻¹ denatured salmon sperm DNA²², the denatured radioactive probe solution was added to the filters in a final concentration of 20 µg ml⁻¹. The filters were incubated for 24–40 h at 75 °C and were dried and exposed to X-ray film (Fuji films Rx Medical) after 4 or 5 serial washes (3 SSC, 0.5% SDS at 75 °C). The incubation temperature used in these experiments was therefore more stringent than the standard conditions used for the experiments shown in Figs 2 and 3, where the filters were incubated at 68 °C. A, pTi-ACH5 *HindIII* restriction pattern (lane a). Lane b represents the autoradiogram of the filter prepared from (a) and hybridised with ³²P-labelled pTi-C58 DNA. B, pTi-C58 *EcoRI* restriction pattern (lane c). Lane d represents the autoradiogram of the filter, prepared from (c) and hybridised with labelled pTi-ACH5 DNA. The numbers indicate the fragments showing the strongest hybridisation between the two types of plasmids.

Fig. 2 Characterisation of the homologous sequence between octopine and nopaline plasmids. The restriction digests, blotting of the DNA fragments to nitrocellulose filters and hybridisations were carried out as described in the legend to Fig. 1. A, pTi-C58: *EcoRI* (a), *SmaI* (c) and *HindIII* (e) restriction patterns, transferred to nitrocellulose filters and respectively (b, d, f) hybridised with the labelled pTi-ACH5 *HindIII* fragment 1 probe. This probe (pGV0201) was in fact a hybrid plasmid constructed by insertion of pTi-ACH5 *HindIII* fragment 1 in the vector pBR322 (ref. 23). B, pGV3505 (pTi-K14): *EcoRI* (a), *SmaI* (c) and *HindIII* (e) restriction patterns, transferred to nitrocellulose filters and respectively (b, d, f) hybridised against labelled pTi-ACH5 *HindIII* fragment 1 probe.



obtained by digestion with *HindIII* showed hybridisation with pTi-C58 DNA.

However, the stability of the hybrids formed by different regions varied considerably (data not shown). These results indicate that the two types of Ti-plasmids diverged from each other, conserving some regions more strictly than others.

To determine more precisely the extent of the homology in the fragments pTi-ACH5 *HindIII*-1 and pTi-C58 *EcoRI*-1, with which the dominant hybridisation was observed, the *HindIII* fragment 1 was cloned (G. De Vos *et al.*, in preparation), labelled and hybridised against the *EcoRI* restriction fragments of pTi-C58. As only the *EcoRI*-1 band of pTi-C58 became radioactive after hybridisation (Fig. 2), it must contain all the DNA homologous to the pTi-ACH5 *HindIII* fragment 1. Furthermore, hybridisation against *SmaI* and *HindIII* restriction fragments of the same pTi-C58 DNA indicated that the *HindIII* fragment 1 of the octopine Ti-plasmid was at least partly homologous to the *SmaI* fragments 5 and 7 (Fig. 2 A, c and A, d) as well as to the *HindIII* fragments 22 (2×10^6 daltons) and 31 (1.1×10^6 daltons) of the nopaline Ti-plasmid C58 (Fig. 2, A, e and A, f). *HindIII* fragments with an identical size and belonging to this conserved region were also found in pTi-K14 (Fig. 2 B, e and B, f) and pTi-T37. A more precise estimate of the size of this 'conserved' DNA sequence can be derived from the sum of the two *HindIII* fragments (together 3.1×10^6 daltons) of the nopaline Ti-plasmid, that hybridised with the pTi-ACH5 *HindIII*-1 fragment. A comparison of the physical maps of

octopine and nopaline Ti-plasmids in the region of the 'conserved' DNA segment (see Fig. 4) shows that the homology between the *HindIII* fragments 22 and 31 of the C58 pTi-plasmid and the *HindIII* fragment 1 of the ACH5 plasmid is restricted to the left end of this fragment. The remaining right part (4.9×10^6 daltons) of the pTi-ACH5 *HindIII* fragment 1 does not show any homology with the DNA of nopaline Ti-plasmids.

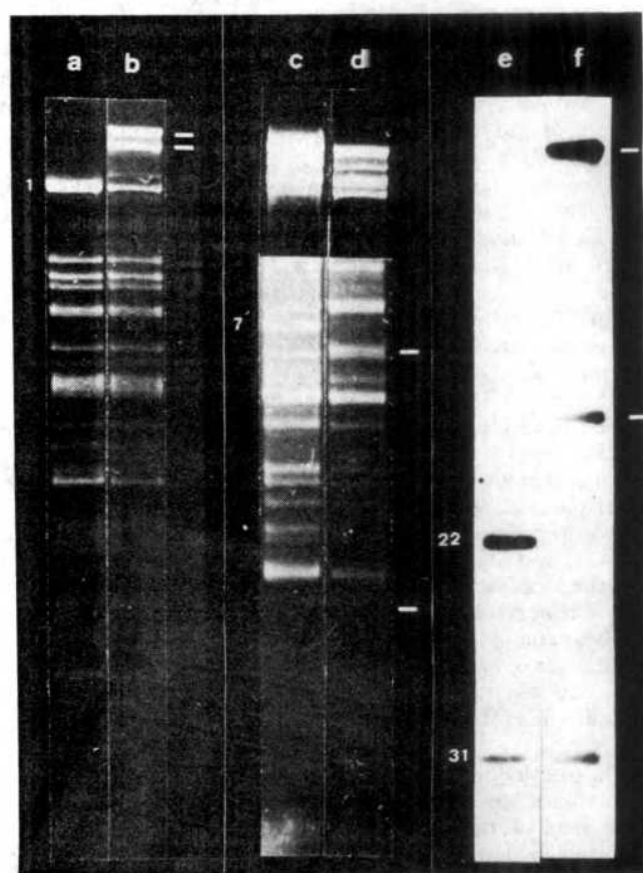
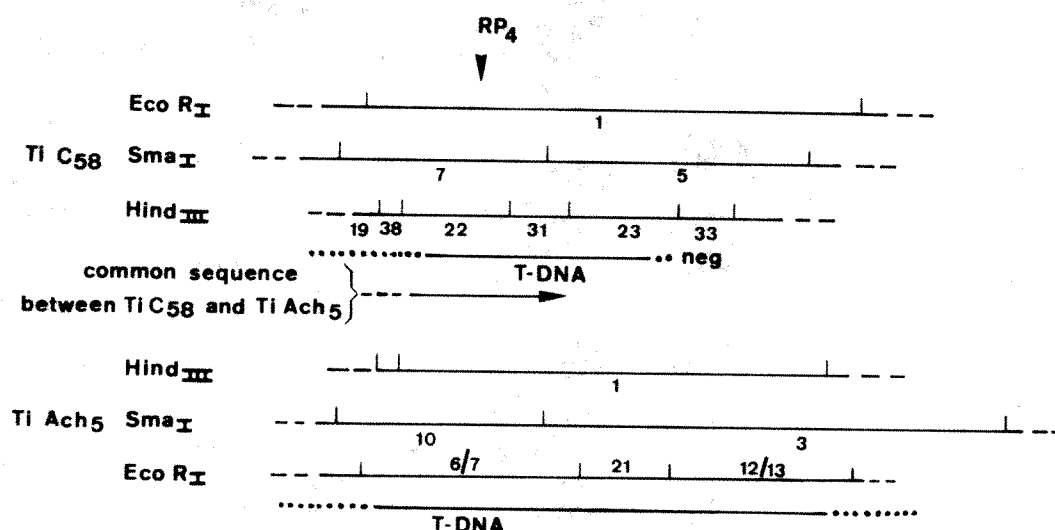


Fig. 3 Localisation of the RP4 integration site in the conserved DNA of the non-oncogenic pTi-C58::pRP4 cointegrate pGV4000. The restriction patterns and hybridisations were carried out as described in the legend to Fig. 1. The pTi-C58 and pGV4000 *EcoRI* (a,b) and *SmaI* (c,d) restriction patterns are shown in parallel to each other. In lanes a and c, the pTi-C58 fragment, in which RP4 is inserted, is indicated by its number. The composite fragment, provided by the junction between pTi-C58 and pRP4 DNA in pGV4000, are indicated by dashes. Lanes a and f, respectively, show the autoradiograms of the pTi-C58 and pGV4000 *HindIII* restriction patterns, which are transferred to nitrocellulose filters and hybridised with the labelled pTi-ACH5 *HindIII* fragment 1 and cloned in the vector pBR322 (ref. 23). The band indicated by an asterisk in lane e is the result of a partial digestion.

Fig. 4 Schematic representation of the conserved region in octopine (pTi-ACH5) and nopaline (pTi-C58) plasmids, showing the restriction map of the corresponding regions in both types of Ti-plasmid. Only those restriction sites used in the present analysis are shown. The cloning, detailed restriction map of the pTi-C58 *EcoRI* fragment 1 and the evidence for the precise localisation of the RP4 integration site in pGV4000 will be described elsewhere (M. De Wilde *et al.*, in preparation). The T-DNA is defined as that segment of the Ti-plasmid that has

been found integrated in the DNA of transformed plant cells. Arguments for the extent and localisation of the octopine T-region and for the relevant restriction map were taken from Chilton *et al.*^{16,24} and were adapted according to instructions from M.-D. Chilton. Arguments for the extent and localisation of the nopaline T-region were obtained in our laboratory and will be described elsewhere (De Beuckeleer *et al.*, in preparation).



The results described by Chilton *et al.*²⁴ indicate that the conserved region extends for another 3.5×10^6 daltons to the left of the *HindIII* fragment 1 of the octopine Ti-plasmids.

To discover whether or not this conserved DNA segment was involved in the oncogenic properties of the Ti-plasmids, we used our previous observations¹⁹, that the integration of a complete P-type plasmid pRP4 into the pTi-C58 plasmid can result in a loss of oncogenicity. Dissociation of the non-oncogenic pTi-C58::RP4 cointegrate (pGV4000) into its Ti and RP4 components resulted in a recovery of the oncogenic properties¹⁹. These observations can be explained if one assumes that pRP4 was reversibly integrated into a pTi-DNA segment that is essential for oncogenicity. The *EcoRI* and *SmaI* restriction fragment patterns of pGV4000 indicated that pRP4 had been integrated into the *EcoRI*-1 and the *SmaI*-7 fragments of pTi-C58 (Fig. 3).

To confirm that the integration of pRP4, resulting in a loss of oncogenicity, had occurred in the 'common' DNA sequence, the pTi-ACH5 *HindIII* fragment 1 was hybridised with the *HindIII* restriction fragments of pTi-C58 and pGV4000 DNA. Figure 3 e and f shows that the integration of pRP4 has occurred into the *HindIII* fragment 22 of the pTi-C58, as this fragment is absent and gives rise to two pTi-C58::RP4 composite DNA fragments. As this fragment 22 is completely homologous to the corresponding pTi-ACH5 DNA, one can conclude that RP4 has indeed been integrated into the conserved segment. A more precise mapping of the pRP4 insertion site (data not shown) has confirmed its localisation within the DNA region homologous to all Ti-plasmids tested so far (see also Chilton *et al.*²⁴). Figure 4 shows the position of the *SmaI*, *EcoRI* and *HindIII* fragments of pTi-C58 and pTi-ACH5 in the region of the conserved segment and the localisation of the pRP4 integration site in pGV4000. The T-region is defined as that segment of the Ti-plasmid that has been found integrated into the plant DNA. The extent and localisation of the 'octopine' T-DNA, determined by *Cot* analysis¹⁶ and the 'nopaline' T-DNA, determined by Southern hybridisation (M. De Beuckeleer, in preparation), can be seen in Fig. 4.

The results presented here indicate that different types of Ti-plasmids have a highly conserved DNA segment and that at least part of this conserved or common DNA segment is involved in determining the oncogenic properties of the Ti-plasmids, as the reversible integration of the pRP4 into this

conserved region results in the reversible loss of oncogenicity. The suggestion of the involvement of this conserved segment in oncogenicity is further supported by the finding that it borders and partly overlaps the regions of both the octopine and the nopaline Ti-plasmids (T-DNA) shown to be present in transformed plant cells¹⁶ (De Beuckeleer *et al.*, in preparation). Some of these results have been previously reported in a preliminary form^{10,15}.

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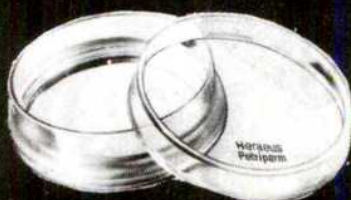
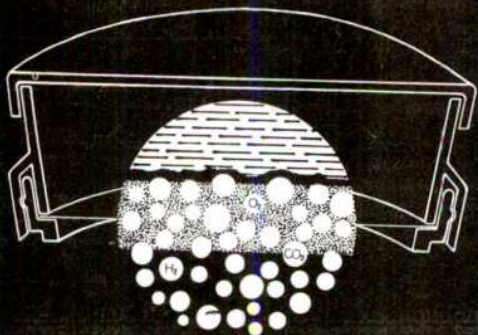
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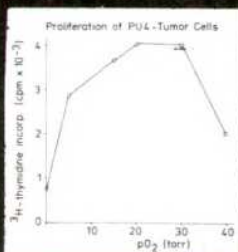
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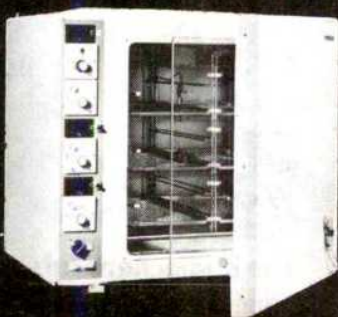
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Different cap 1:cap 2 ratios in rabbit α and β globin mRNA

CAP structures type 0 ($m^7G^{5'}ppp^5X$), type 1 ($m^7G^{5'}ppp^5XmY$), and type 2 ($m^7G^{5'}ppp^5XmYmZ$), differing in the number of 2'-O-methylated penultimate nucleotides, have been found at the 5' termini of many viral and non-viral eukaryotic messenger RNAs¹. The ability to quantitatively remove the $m^7G^{5'}ppp$ -blocking group from eukaryotic mRNAs and to subsequently label their 5' termini with ^{32}P using [γ - ^{32}P]ATP and T_4 polynucleotide kinase has led to the determination of nucleotide sequences present at the 5' termini for rabbit α and β globin mRNA^{2,3} and alfalfa mosaic virus RNA 4 (ref. 4). The first six nucleotides of both rabbit α and β globin mRNA are identical and these two mRNAs each contain a mixture of cap types 1 ($m^7G^{5'}ppp^5m^6AmC$) and 2 ($m^7G^{5'}ppp^5m^6AmCmA$), differing only by the 2'-O-methylation of their penultimate C residues². This 5'-end labelling procedure can also be used to determine the relative amounts of cap types for each individual rabbit

print both on the basis of size and base composition². Further limited digestions of each 5'- ^{32}P -labelled T_1 -fragment with either pancreatic ribonuclease (cleaving only after pyrimidines and yielding ^{32}P - m^6AmCp and ^{32}P - $m^6AmCmAcp$) or T_2 ribonuclease (cleaving after both pyrimidines and purines and yielding ^{32}P - m^6AmCp and ^{32}P - m^6AmCmA) were carried out to determine the relative amounts of type 1 and type 2 cap structures present in each mature mRNA molecule.

Figure 1 shows a representative two-dimensional homochromatographic analysis of a pancreatic RNase digest of the ^{32}P - β -globin mRNA 5'-terminal T_1 -fragment purified as described above. The two distinct products which are clearly resolved in this system, ^{32}P - m^6AmCp and ^{32}P - m^6AmCmA , are separated on the basis of both size and base composition⁵. By similar analysis, a T_2 -RNase digest of the same β - T_1 -fragment also resolved two products, ^{32}P - m^6AmCp and ^{32}P - m^6AmCmA (data not shown). By quantitating the radioactivity present in each product, one can determine the cap 1:cap 2 ratio for an individual globin mRNA. The results from various preparations of rabbit α and β globin mRNA differing only in their relative recovery are summarised in Table 1. The cap 1:cap 2 ratio in three different mRNA preparations was consistently two- to threefold higher for rabbit α mRNA than for β mRNA. This result was independent of the relative quantities of α and β mRNA present in various preparations and the method used for separation and measurement of the T_2 or pancreatic digestion products. Also, these results did not reflect a difference in a potentially lower percentage of 5'-labelling of a type 2 cap structure by T_4 polynucleotide kinase due to the additional 2'-O-methylation⁶, as this would have been the case for both α and β mRNAs.

The significance of a two- to threefold enrichment of type 1 cap structures in mature rabbit α relative to β globin mRNA is

Table 1 Ratio of c.p.m. cap 1/c.p.m. cap 2 in [$5'$ - ^{32}P]-labelled rabbit α and β globin mRNA

Preparation	α/β	Analysis				Mean ratio cap 1/cap 2
		T_2 digest		Pancreatic digest		
		A	C	B	C	
I	0.53	α 515/230 (2.24)	—	—	—	2.24
		β 503/420 (1.20)	—	—	—	1.20
II	1.10	α 650/200 (3.24)	2,200/650 (3.40)	770/275 (2.80)	—	3.15
		β 440/320 (1.37)	—	440/295 (1.35)	700/660 (1.06)	1.27
III	0.35	α —	1,390/530 (2.62)	—	1,210/390 (3.10)	2.86
		β —	2,000/1,425 (1.40)	—	2,950/1,735 (1.70)	1.55

Limit digestion of ^{32}P -labelled α and β globin mRNA 5'-terminal T_1 fragments with either T_2 or pancreatic RNase were analysed as follows. A, Thin layer partition chromatography in isobutyric acid-concentrated NH_4OH-H_2O (66:1:33, v/v) (pH 3.7). B, Thin layer partition chromatography in 0.1 M sodium phosphate (pH 6.8) (100 ml) $(NH_4)_2SO_4$ (60 g), and *n*-propanol (2 ml) C, Two-dimensional homochromatography as described in Fig. 1. All three methods of analysis completely resolved both cap 1 and cap 2 structures from T_2 and pancreatic RNase digestions. Thin layer or homochromatography plates were autoradiographed and material detached from the glass by either scraping or removed as solid platelets using a solution of nitrocellulose⁷ and then counted in toluene scintillant⁵. α/β mRNA ratios were determined from counts present in both ^{32}P -labelled α and β 5'-terminal T_1 fragments purified by two-dimensional fingerprinting² of a T_1 RNase digest of unfractionated 5'- ^{32}P -labelled globin mRNA. There was reproducibly a direct correspondence between the relative amounts of radioactivity present in each 5'- ^{32}P -labelled T_1 fragment and the actual relative amounts of α and β globin mRNA present in each mRNA preparation as determined by polyacrylamide gel electrophoresis in both 7 M urea and 98% formamide². Differences in relative recoveries for α and β mRNA in various polysomal RNA preparations were due to procedural variations during phenol extraction of reticulocyte polysomes².

globin mRNA, without having to first purify each mRNA to homogeneity². In this work the cap 1:cap 2 ratios have been determined. The results indicate a two- to threefold enrichment of type 1 cap structures in α relative to β mRNA.

When a mixture of [$5'$ - ^{32}P]-labelled α and β globin mRNA was completely digested with T_1 ribonuclease, the individual α and β [$5'$ - ^{32}P]-labelled T_1 fragments were purified by two-dimensional fingerprintings². The α - T_1 -fragment (^{32}P - $m^6AmC(m)ACUUCUGp$) and the β - T_1 -fragment (^{32}P - $m^6AmC(m)ACUUCGp$) resolve from one another in the finger-

not clear. Results obtained by incubating erythropoietic mouse spleen cells with [methyl- ^3H]methionine have suggested that newly synthesised α and β globin mRNA may also exhibit similar cap ratio differences⁸. In the reticulocyte it has been estimated that each molecule of β globin mRNA initiates protein synthesis at a rate 30% faster than α mRNA⁹⁻¹¹. This difference is probably reflected in a difference in their nucleotide sequences at or near the region for initiation of protein synthesis. In this laboratory, it has been shown that there is a strict requirement for the integrity and presence of the m^7G

residue in the 5'-terminal cap structure for translation of rabbit globin mRNA both *in vitro* and *in vivo*¹². It has also been shown that β -eliminated vesicular stomatitis virus and reovirus mRNA have significantly higher rates of ribosome binding than corresponding unmethylated mRNAs in a reticulocyte lysate¹³. Whether the relative amounts of secondary 2'-O-methylations in normally capped α and β globin mRNA help determine their respective ribosome binding rates in the reticulocyte, however, is open to speculation.

In both mouse L cells¹⁴ and Novikoff hepatoma cells¹⁵, it has been shown that cap structures in cytoplasmic mRNA are derived from type 1 cap structures in heterogeneous nuclear RNA (hnRNA) from which a significant proportion is gradually converted to type 2 cap structures in the cytoplasm. Whether the observed differences in amounts of secondary 2'-O-methylations in rabbit α and β globin mRNA are therefore a reflection of their relative age or relative rates of mRNA turnover during erythroid development is currently under investigation. Recent experiments with mature mouse α and β globin mRNA also indicate similar cap 1:cap 2 ratio differences.

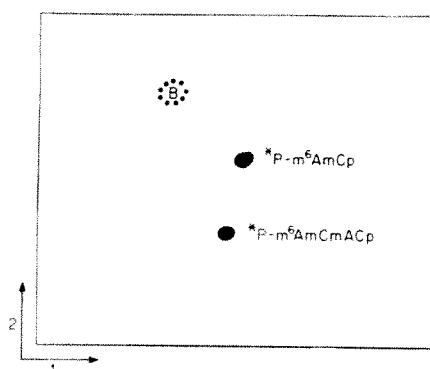


Fig. 1 Autoradiogram of a pancreatic RNase digest of the 32P- β globin mRNA 5'-terminal T₁-fragment. First dimension: cellulose acetate electrophoresis in pyridinium acetate buffer (pH 3.5) in 7 M urea. Second dimension: DEAE-cellulose TLC in 50 mM KOH strength 'homomix'⁵. The circled 'B' indicates the position of the xylene cyanole FF dye. Preparation of ³²P-labelled α - and β -globin mRNA 5'-terminal T₁ fragments was as described elsewhere². Pancreatic and T₂ RNase limit digestion conditions of these fragments and their subsequent analysis by two-dimensional homochromatography has also been described^{2,5}.

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The murine kappa light chain shift

THE immunoglobulins of mice and other animals consist of several distinct heavy chains associated with either of two light chains (κ or λ). Adult animals of different species produce immunoglobulins which demonstrate widely divergent ratios of κ to λ light chains. In mice, most of B-cell surface immunoglobulins possess κ light chains¹. However, as all previous work on the ratio of κ/λ immunoglobulins was based on experiments with adult animals, the possibility that immature populations of B cells may demonstrate a more evenly balanced ratio of κ to λ positive cells was investigated. In this study, populations of cells known to contain immature B lymphocytes were examined by indirect immunofluorescence for the presence of κ and λ positive cells. The results demonstrate that fetal liver, bone marrow and spleens from young mice contain B cell populations with a κ/λ ratio approaching unity. In the spleen, the κ/λ ratio increased as a function of age with the most dramatic shift occurring between 4 and 6 weeks of age. A similar, but less dramatic increase in the κ/λ ratio occurs in the bone marrow. Based on these data, we propose that the final predominance of κ -bearing cells is based on a two-stage process. Initially, there is an equal probability of κ or λ light chain expression at the cell surface. However, the κ -bearing cells, which possess a more abundant repertoire of V_κ genes, eventually predominate as a result of antigen driven clonal expansion.

Ontogenetic shifts occur between different heavy chain constant region (C_H) genes, allowing the association of the same V region genes with various C_H genes²⁻⁷. In all cases studied, these genes determining the various heavy chains exist in a single linkage group and share a common set of variable region (V_H) genes⁸⁻¹⁴. By contrast, the two classes of light chain, κ and λ , are determined by unlinked genes and each is associated with its own characteristic set of V genes¹⁵. There is, as yet, no evidence of any relationship between light chain class and physiological function or of any ontogenetic shift between λ and κ light chains. Hood *et al.*¹ have suggested that the predominance of κ or λ in a species is due to an evolutionary selection process in which a positive force has preferentially favoured one class of light chain.

It has been observed that the proportion of plasmacytomas and myelomas expressing κ or λ light chains in mice reflects the frequency of those light chains found in normal serum immunoglobulins^{16,17}. Further, it has been observed that the more abundant class of light chain in each species is associated with a greater number of V region subgroups, presumably reflecting a greater number of associated germ-line V genes¹⁶. These observations suggest that the imbalance in the κ/λ ratio is proportional to the number and diversity of the V region genes associated with each.

Here we present evidence that the expression of either κ or λ at the cell surface is a two-stage process. The initial choice between expression of κ or λ is based purely on random selection of an equal number of constant region genes. The preferential display of one or the other is then most likely based on antigen driven clonal selection, which in turn is

determined by the number of κ and λ V genes. Furthermore, we believe this selection force occurs during ontogeny, rather than as a phylogenetic event.

The percentage of cells in the spleen, bone marrow, and fetal liver which bear surface κ and λ light chains has been determined by indirect immunofluorescence. As shown in Table 1, the absolute percentage of Ig-positive cells increases in the spleen as a function of age; however, the ratio of κ - to λ -positive cells does not remain constant during this process. Initially, in the spleen of 1-week-old mice, the ratio (κ/λ) is 2.03, yet by 6 weeks of age this ratio has reached 6.59. The shift is not gradual as one might expect, but dramatically changes between 4 and 6 weeks of age. This shift seems to be T-cell independent in that the κ/λ ratio in adult nude mice does not significantly differ from normal adult mice (data not shown). By contrast, the κ/λ ratio in the bone marrow remains near unity until 1 yr of age when the value increases slightly. Similarly, the κ/λ ratio of Ig-positive cells in the fetal and newborn liver approaches unity. A plot of the κ to λ ratio in

the bone marrow and spleen as a function of age is shown in Fig. 1.

Statistical analysis of these data confirms the significance of the apparent increase in the κ/λ ratio (Table 2). In the spleen population, a clear pattern emerges. The κ/λ ratios of animals 1, 2 and 4 weeks old are not statistically different, however, the κ/λ ratios of the 6-week and 1-yr-old mice do differ from the 1-4-week-old groups. Although the κ/λ ratio in the spleen seems to increase again between 5 weeks and 1 yr, the difference was not significant ($P > 0.05$). The differences in the ratios of the spleen populations have been further confirmed by non-parametric Mann-Whitney U -sample and one-way analysis of variance testing (see Table 2, legend). The results from these statistical analyses and the apparent trend for the κ/λ ratio in the spleen to increase as a function of age (Fig. 1) support our hypothesis that the κ/λ ratio shifts during ontogeny as a result of some positive selection force, possibly antigenic stimulation. Analysis of the results from the bone marrow population also suggest an increase in the κ/λ ratio as a

Table 1 % κ -positive and λ -positive cells in lymphoid organs as a function of age

Age	Cell population	No. tested	% Stained cells/Total nucleated cells*			Ratio $\kappa:\lambda$
			R α Mlg	R $\alpha\kappa$	R $\alpha\lambda$	
Fetal (18-19d)	Liver	3 pools†	16.7 $\pm$ 1.5‡ (15-18)	10.3 $\pm$ 2.1 (8-12)	9.0 $\pm$ 2.0 (7-11)	1.14
	Thymus	3 pools	2.3 $\pm$ 0.6 (2-3)	ND	ND	
Newborn (<24 hr)	Liver	3 pools	16.3 $\pm$ 2.3 (15-19)	7.7 $\pm$ 2.5 (5-10)	9.3 $\pm$ 3.2 (7-13)	0.83
	Thymus	4 pools	2.8 $\pm$ 0.5 (2-3)	ND	ND	
1 week	Spleen	3 pools	14.0 $\pm$ 2.0 (12-16)	9.7 $\pm$ 0.6 (9-10)	4.7 $\pm$ 1.2 (4-6)	2.06
	Thymus	3 pools	2.0 $\pm$ 1.0 (1-3)	ND	ND	
2 weeks	Spleen	4	20.5 $\pm$ 6.9 (11-27)	14.0 $\pm$ 4.1 (10-18)	4.0 $\pm$ 1.4 (3-6)	3.50
	Thymus	4	1.0 $\pm$ 0.8 (<1-2)	ND	ND	
	Bone marrow	3 pools	24.3 $\pm$ 3.5 (21-28)	8.3 $\pm$ 2.1 (6-10)	13.0 $\pm$ 5.6 (7-18)	0.64
4 weeks	Spleen	5	42.6 $\pm$ 1.5 (41-45)	33.4 $\pm$ 5.8 (26-40)	11.0 $\pm$ 4.1 (6-15)	3.03
	Thymus	4	0.3 $\pm$ 0.5 (<1-1)	ND	ND	
	Bone marrow	4	22.8 $\pm$ 4.4 (19-29)	10.5 $\pm$ 1.9 (9-13)	11.3 $\pm$ 4.8 (7-18)	0.93
6 weeks	Spleen	4	51.8 $\pm$ 5.2 (48-59)	45.5 $\pm$ 5.3 (41-53)	6.9 $\pm$ 1.3 (5-8)	6.59
	Thymus	4	1.3 $\pm$ 1.0 (<1-2)	ND	ND	
	Bone marrow	5	25.6 $\pm$ 2.8 (21-28)	12.8 $\pm$ 3.0 (8-16)	10.2 $\pm$ 5.3 (4-17)	1.25
1 yr	Spleen	8	49.4 $\pm$ 3.0 (46-54)	44.5 $\pm$ 3.9 (41-52)	5.5 $\pm$ 2.3 (3-9)	7.95
	Thymus	4	2.0 $\pm$ 1.4 (1-4)	ND	ND	
	Bone marrow	4	26.8 $\pm$ 5.0 (22-32)	18.5 $\pm$ 6.4 (13-25)	9.3 $\pm$ 3.0 (6-13)	1.99

* Indirect immunofluorescence was performed by a modification²¹ of the method of Pernis *et al.*²². Rabbit anti- κ chain serum (R $\alpha\kappa$) was from Bionetics and rabbit anti- λ chain serum (R $\alpha\lambda$) was provided by Dr James D. Folds (UNC, Chapel Hill). Polyspecific rabbit anti-mouse immunoglobulin serum (R α Mlg) was produced as described previously²³. Fluorescein-conjugated goat anti-rabbit Ig was from Meloy Laboratories. All antisera were used at a dilution of 1:40 in PBS-0.05M Na₂S₂O₅. Controls for phagocytosis and nonspecific adherence of Ig consisted of cells incubated only with the second antiserum or by using normal rabbit serum in place of the primary antiserum. These were consistently found to be negative (<2%). Results are expressed as % cells positive for surface fluorescence/total nucleated cells counted (>200). Mice of the C57BL/10ScSn and CBA/J background were used. As there was no apparent strain differences all data were pooled for analysis.

† A 'pool' consisted of the spleens, thymuses, or bone marrows of 4 animals which were combined in order to obtain sufficient cells to perform the assay.

‡ Mean $\pm$ standard deviation and range of percentages obtained from determinations for each cell population. ND, not determined.

Table 2 Statistical analysis of change in κ/λ ratio in splenic and bone marrow B lymphocytes during ontogeny: paired contrast *t*-testing

	Age	No. of observations	$\bar{R}$	Statistical difference from:				
				1 week	2 weeks	4 weeks	6 weeks	1 yr
Spleen	1 week	3 pools	2.14 ± 0.43*	X				
	2 weeks	4 pools	3.83 ± 1.64	NS†	X			
	4 weeks	5 pools	3.54 ± 1.91	NS	NS	X		
	6 weeks	4 pools	6.92 ± 1.42	<i>P</i> < 0.005	<i>P</i> < 0.025	<i>P</i> < 0.01	X	
	1 year	8 pools	9.25 ± 4.1	<i>P</i> < 0.0005	<i>P</i> < 0.0005	<i>P</i> < 0.0005	NS	X
Bone marrow	2 weeks	3 pools	0.62 ± 0.31	—	X			
	4 weeks	4 pools	1.01 ± 0.28	—	NS	X		
	6 weeks	5 pools	1.53 ± 0.86	—	NS	NS	X	
	1 year	4 pools	2.07 ± 0.65	—	<i>P</i> < 0.025	<i>P</i> < 0.025	NS	X

Spleen and bone marrow cells from mice of various ages were assayed for % κ -positive and % λ -positive cells and κ/λ ratio was computed for each observation. For statistical analysis, the mean of the individual κ/λ ratios was determined for each age category. This convention was chosen since comparing the ratios of means is a less well-established procedure for this type of multi-level experiment. For parametric testing it is assumed that these observations are normally distributed as there is no apparent reason to indicate otherwise. Results from the paired contrast *t* test²⁴ are presented above. Results were also tested for significance by one-way analysis of variance (ANOVA)²⁴ and non-parametric Mann-Whitney *C*-sample testing²⁵. On ANOVA testing, the means of the κ/λ ratios in the different age categories of the spleen were found to be statistically different (*P* < 0.001). Similarly when tested by the more conservative non-parametric Mann-Whitney *C*-sample test, these ratios were also found to differ (*P* < 0.001). Although the paired contrast *t* test demonstrates significance for the different age groups in the bone marrow, the means of the bone marrow age groups were not different (*P* > 0.05) when tested by ANOVA and Mann-Whitney *C*-sample testing. Further, it should be noted that these cell populations do not demonstrate homoscedasticity (equal dispersion within age categories), thus the results from these statistics may be affected by failure to comply with this assumption.

* Mean of the individual κ/λ ratios ± s.d.

† Not significant at α = 0.05.

function of age. When the means of the ratios for the different age categories were compared by paired contrast *t*-testing, the 2, 4 and 6-week-old groups were not different, however the 1-yr-old group was found to be different from the 2 and 4-week-old groups. Unlike the results obtained with the spleen populations, we were unable to confirm the significance of the changes in the bone marrow with one-way analysis of variance and non-parametric Mann-Whitney *C*-sample testing. Despite these limitations, a general trend towards an increasing κ/λ ratio is seen in the bone marrow as a function of age, suggesting a κ/λ shift occurs which is similar to, but less dramatic than, the shift seen in the spleen.

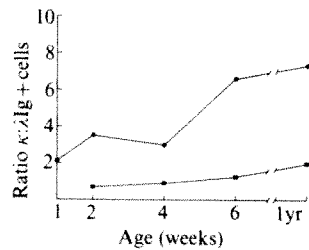


Fig. 1 Shift in κ/λ ratio in murine B lymphocytes with age.

Barandun *et al.*¹⁸ have also reported a slight but significant shift in the κ/λ ratio in normal human serum immunoglobulins as a function of age. Also similar to our findings, Spear *et al.*¹⁹ have shown a near doubling in the percentage of Ig-positive Swiss-L mice spleen cells between 2 and 4 weeks of age. Coincident with the increase in B cells, they noted an onset of the capacity to respond to antigens and a significant increase in the ratio of B to T lymphocytes. These events are thought to occur as a result of, rather than the cause for, immunological competence.

Based on these data, we propose that prior to an antigen driven selection process the probability of a cell expressing

surface κ or surface λ is equal. The nearly equal proportion of κ - and λ -positive cells in the populations which contain immature B cell precursors (bone marrow and fetal liver), strongly supports this hypothesis. Between 2 and 4 weeks of age an overall increase in the percentage of Ig-positive spleen cells occurs, yet the relative κ/λ ratio remains essentially the same. However, between 4 and 6 weeks of age, this ratio increases significantly (Table 2). This event may be due to the onset of immunological competence in the animal, allowing the selective clonal expansion of κ -bearing B cells. Molecular studies of immunoglobulin diversity also support this idea. In mice, which have so many more *V* genes associated with and available as partners to the κ *C* gene, the probability that they can collaborate with *V_H* genes to provide a good 'fit' for antigen is high¹⁶. Thus, during maturation, exposure and response to antigens will favour the expansion of the clones of cells expressing κ light chains.

As a corollary to our hypothesis, we would predict that the examination of spleen cells of adult gnotobiotic mice would show a κ/λ ratio significantly different from their normal littermates (that is, closer to unity). Furthermore, it may be possible to shift the κ/λ imbalance prematurely by deliberate immunisation of 2–3-week-old mice. Data from such experiments have proven suggestive, but not significant. The injection of a few exogenous antigens may be inconsequential in relation to the vast array of endogenous and environmental antigens 'seen' by the animal when immunological competence occurs.

The stimulus for this study was the observation that two recently discovered murine B-cell lymphomas were both found to express λ light chain associated with their surface IgM²⁰. This seemed to be most unlikely, on the basis that only about 5% of mouse immunoglobulins express λ chains and only very few murine myelomas have λ light chains^{16,17}. However, other cell surface characteristics (the absence of IgD and C'3 receptors) suggested that both tumours had been derived from a population of cells early in the ontogenetic pathway of B cells²⁰. Thus, we were led to examine other populations of immature B cells. Having done so, it seems that the initial selection made during maturation is between κ and λ *C* genes, and that only later do *V* genes become influential (that is, at

the time of onset of immunological competence). As a result, we further predict that in all species, the proportion of malignancies of 'early' B cells expressing surface κ or λ light chains will approach unity.

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Comparison of the evolution rates of cytosolic and mitochondrial aspartate aminotransferase

TWO isoenzymes of aspartate aminotransferase (AAT) occur in animal and possibly in all eukaryotic cells, one in the cytosol (cAAT) and the other in the mitochondria (mAAT)¹. Both isoenzymes are composed of two identical polypeptide chains of about 400 amino acid residues and their reaction mechanisms and kinetic features are essentially the same^{1,2}. They are homologous proteins, as evidenced by 48% sequence identity of the two isoenzymes from pig^{3,4}, and are both coded for by nuclear DNA and synthesized by cytosolic ribosomes⁵⁻⁷. Comparison of the isoenzymes from pig with those from chicken by quantitative microcomplement fixation has shown that the interspecies similarity of the mitochondrial isoenzymes markedly exceeds that of the cytosolic isoenzymes⁸. The same conclusion has been reached by comparison of the known amino acid sequences of the pig isoenzymes^{3,4} with the chicken isoenzymes, for which more than half of their sequences have already been determined. The sequence identity of the two cytosolic isoenzymes seems to be approximately 50% (Y. M. Torchinsky, personal communication), whereas that of the two

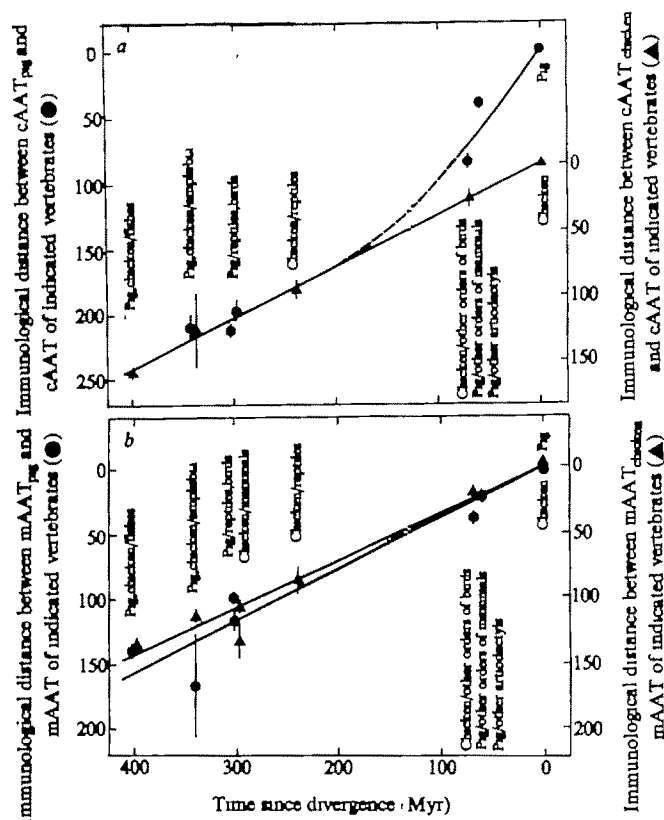


Fig. 1 Evolution of cytosolic (a) and mitochondrial (b) AAT. The mean immunological distances between the reference enzymes from pig and from chicken and the respective isoenzymes from the indicated vertebrate classes or orders are plotted against the palaeontologically estimated times of divergence of the ancestors of the reference species pig and chicken from the various vertebrate classes or orders. Immunological distances are taken from Table 1 and are not corrected for multiple mutations at the same site. To join the lines of the cytosolic pig and chicken enzyme in a at the point of their divergence 300 Myr ago, the line of the porcine enzyme was translated vertically by +53 immunological distance units. This translation distance was determined as the difference between the immunological distance of mammals and birds (mean of the reciprocal crossreactions 200 units) and the interpolated value of the line of the chicken enzyme at 300 Myr (117 units). The assumed times of divergence are¹³, in Myr: fishes, 400; amphibians, 340; birds and reptiles from mammals, 300; birds from reptiles, 240; interordinal divergence of placental mammals, 70; interordinal divergence of birds, 70; pig from the other artiodactyls, 60. The slope of the lines corresponds to the double value of the rate of evolution because the immunological distance measured is the sum of the changes accumulated by the 2 species compared since divergence from a common ancestor. The slope of the line of cAAT is 0.39 ($r = 0.95$) throughout the vertebrate classes except for mammals, where the slope is 0.97. For mAAT, the slope is 0.39 ($r = 0.96$) and 0.36 ($r = 0.98$) when measured with anti-mAAT_{pg} antiserum and with anti-mAAT_{chicken} antiserum, respectively.

mitochondrial isoenzymes is about 85% (ref. 9 and U. Hausner, P. C., K. J. Wilson, unpublished). In the present study, the immunological interspecies comparison of the two isoenzymes was extended to species of all vertebrate classes. The evolution of the two compartmented isoenzymes was found to have proceeded at identical and constant rates throughout the development of the nonmammalian vertebrates. After the emergence of mammals, however, the rate of evolution of the cytosolic isoenzyme more than doubled while its mitochondrial counterpart retained the slower rate of the nonmammalian vertebrates.

Table 1 Immunological distance between cAAT and mAAT from pig and chicken and the respective isoenzymes from species of all vertebrate classes

Species (source of extract)	Immunological distance between reference enzyme and respective isoenzyme from the indicated species			
	cAAT _{pig}	mAAT _{pig}	cAAT _{chicken}	mAAT _{chicken}
Artiodactyls				
Pig (<i>Sus scrofa</i>)	0	0	218	108
Black-Buck (<i>Antelope cervicapra</i>)	45	30	177	120
Sheep (<i>Ovis ammon</i>)	33	22	162	98
Ox (<i>Bos taurus</i>)	30	13	178	102
Stag (<i>Cervus elaphus</i>)	41	24	186	100
Alpaca (<i>Lama guanacoë</i>)	49	28	187	110
Mean $\pm$ s.e.m.	40 $\pm$ 4	23 $\pm$ 3.5 $\pm$ 8	106 $\pm$ 3	
Species of other mammalian orders				
Dog (<i>Canis familiaris</i>)	63	39	189	116
Cat (<i>Felis silvestris</i>)	73	26	224	116
Horse (<i>Equus caballus</i>)	67	40	232	173
Otter (<i>Lutra lutra</i>)	97	52	237	123
Mean $\pm$ s.e.m.	75 $\pm$ 8	39 $\pm$ 5	221 $\pm$ 11	132 $\pm$ 14
Birds				
Chicken (<i>Gallus gallus</i>)	189	102	0	0
Duck (<i>Anas platyrhynchos</i>)	162	98	12	20
Goose (<i>Anser anser</i>)	180	109	18	18
Rhea (<i>Rhea americana</i>)	197	89	7	28
Pigeon (<i>Columba livia</i>)	236	98	10	12
Budgerigar (<i>Melopsittacus undulatus</i>)	207	105	45	43
Buzzard (<i>Buteo buteo</i>)	215	82	48	13
Crow (<i>Corvus corone</i>)	192	119	42	7
Mean $\pm$ s.e.m.	197 $\pm$ 8	100 $\pm$ 4	26 $\pm$ 7	20 $\pm$ 5
Reptiles				
Tortoise (<i>Testudo hermanni</i>)	224	126	104	107
Turtle (<i>Chelonia</i> spp.)	207	110	100	102
Crocodile (<i>Crocodilus niloticus</i>)	215	100	75	73
Python (<i>Python reticulatus</i>)	203	130	100	63
Mean $\pm$ s.e.m.	212 $\pm$ 5	117 $\pm$ 7	95 $\pm$ 7	86 $\pm$ 11
Amphibia				
Frog (<i>Rana ridibunda</i>)	219	127	98	110
Salamander (<i>Pleurodeles walthii</i>)	198	207	154	113
Mean $\pm$ s.e.m.	209 $\pm$ 11	167 $\pm$ 40	126 $\pm$ 29	112 $\pm$ 2
Fishes				
Whitefish (<i>Coregonus fera</i>)	—	135	151	138
Trout (<i>Salmo fario</i>)	—	151	172	132
Carp (<i>Cyprinus carpio</i>)	—	130	158	143
Grayling (<i>Thymallus thymallus</i>)	—	143	160	128
Mean $\pm$ s.e.m.	—	140 $\pm$ 5	160 $\pm$ 4	135 $\pm$ 3

The immunological distance between the reference antigens (isoenzymes from pig and chicken purified to homogeneity) and the corresponding isoenzymes of the tested species were measured by quantitative microcomplement fixation as reported previously⁸. In pig and chicken the immunological distances obtained with heart tissue extracts were the same as those determined previously with the isolated enzymes⁸. No crossreactions between isolated cAAT and mAAT from either pig or chicken were observed. Thus, heart tissue extracts were tested without separating the two isoenzymes. Heart tissue was frozen not later than 3 h post mortem and stored at -20°C . The thawed tissue was homogenised with a Duall homogeniser in 5 volumes of the buffer used for the complement fixation, and centrifuged at $2,000g$ for 15 min. The extent of complement fixation at different concentrations of antiserum was determined at serial dilutions of the homologous and the heterologous antigen. A plot of the peak heights of the complement fixation curves against the logarithm of the antiserum concentration yielded a straight line. The lines of the crossreactions of a given antiserum with heterologous antigens were essentially parallel to the line obtained for the homologous reaction. The immunological distance (ID) was calculated according to $ID = 100 \log$ (antiserum concentration for 50% complement fixation with the heterologous antigen/antiserum concentration for 50% complement fixation with the homologous antigen) (refs 8, 10). Both anti-mAAT antisera crossreacted with rabbit heart extract as judged by quantitative microcomplement fixation and the Ouchterlony double-diffusion test. The crossreacting antigen from rabbit heart showed the same electrophoretic mobility in immunoelectrophoresis and the same molecular weight as mAAT from pig and chicken (determined by polyacrylamide gel electrophoresis in sodium dodecyl sulphate after extraction of the antigen-antibody complexes with *Staphylococcus aureus* carrying protein A). Thus, it is very likely that the immunisation with either heterologous mitochondrial isoenzyme had resulted in the production of antibodies against the rabbit's own mAAT. Similar breakage of the immunological tolerance has been reported for α -fetoprotein¹¹. In the present instance, this phenomenon might be related to the sequestration of the antigen in the mitochondria. The crossreactions in quantitative microcomplement fixation between the rabbit mAAT and the rabbit antisera against mAAT from pig and chicken are weak, corresponding to an immunological distance of 211 and 206, respectively. These crossreactions reflect an increase in antigenicity of the mitochondrial isoenzymes, that is, a larger surface portion surveyed by the antibodies, which may be expected to increase the reliability of the immunology sequence relationship¹². No reliable crossreactions were obtained from anti-cAAT_{pig} antiserum and the fish heart extracts.

Antisera to cAAT and mAAT from both pig and chicken were produced as described previously⁸. The degree of immunological crossreactivity of these reference antisera with the respective isoenzymes from representatives of all vertebrate classes was determined by quantitative microcomplement fixation and was expressed in terms of immunological distance (Table 1). Remarkably, the immunological distances between cAAT_{pig} and cAAT from all other species are substantially larger than those between mAAT_{pig} and mAAT from the other species. Similarly, the immunological distances between cAAT_{chicken} and cAAT from mammalian species are twice as large as the corresponding immunological distances obtained for mAAT_{chicken}. In contrast, the same immunological distances were obtained when cAAT_{chicken} and mAAT_{chicken} were compared with the corresponding isoenzymes from the non-mammalian vertebrate classes. The temporal course of the evolution of the cytosolic and the mitochondrial isoenzymes is reconstructed in Fig. 1a and b, respectively. The plots of the immunological distances against the times of divergence of the phylogenetic lineages show a constant rate of change for mAAT throughout all vertebrate classes. A constant rate of the same magnitude applies also to cAAT in all nonmammalian vertebrates. However, during the development of mammals, the evolution of the cytosolic isoenzyme proceeded more than twice as fast, indicating that an increase in the rate of change had occurred after the mammals had diverged from the common ancestor of reptiles and birds. This acceleration of the rate of evolution as found with the anti-cAAT_{pig} antiserum is confirmed by the reciprocal reactions with anti-cAAT_{chicken} antiserum (see Table 1).

For a set of homologous variants of a given protein, the immunological distance (ID) has been found to correlate linearly with the percentage amino acid sequence difference (Δaa) according to the equation $ID = 5 \times \Delta aa$ (refs 14, 15). For cAAT, the average time required for a 1% change in the amino acid sequence to arise between two genealogical lineages can thus be estimated to be 6 Myr in mammals and 12 Myr in the other vertebrates. The latter estimate also obtains for mAAT throughout the phylum of vertebrates. The values lie within the range found for other intracellular enzymes¹⁶. On the basis of these rates of change, the gene duplication underlying the development of the mitochondrial and the cytosolic isoenzyme (amino acid sequence difference in pig, 52% (ref. 4)) may be assumed to have taken place about 600 Myr ago, at the time of the emergence of the vertebrates. This indicates that the isoenzymes of the vertebrates do not originate from the same gene duplication that gave rise to the isoenzymes found in fungi¹⁷ and plants¹⁸.

Changes in the rate of evolution of certain proteins such as globin chains¹⁹ or lysozyme/lactalbumin²⁰ have been reported, but such changes have been questioned¹⁶. However, the main argument against these cases, that of the uncertainty in estimating the time of interspecies divergence, does not seem pertinent to aspartate aminotransferase. The increase in rate is found specifically with the cytosolic isoenzyme, whereas the closely related mitochondrial isoenzyme retains the slower rate of evolution. Thus, the conclusion that the cytosolic isoenzyme changed its rate of evolution is warranted independently of the palaeontological time scale.

The increase in the rate of evolution of the cytosolic isoenzyme in mammals may be due to positive natural selection towards an improved, newly developed function of the isoenzyme or may reflect the loss of an obsolete noncatalytic function. The cause of the change in the evolution rate remains open to conjecture. The fact, however, that the crucial difference between the two compartmented isoenzymes exists only in mammals might facilitate its eventual identification. Data soon available on the primary and spatial structure of both isoenzymes²¹⁻²³ will enable the comparative study of the evolution of their structural and functional features, thus providing an informative approach to the study of the interrelationship of function and evolution of proteins.

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Structure of DNA predicted from stereochemistry of nucleoside derivatives

CRYSTALLOGRAPHIC data on the constituents of nucleic acids can be very useful in predicting secondary structures. Until recently only data from the crystal structures of mononucleosides and mononucleotides have been used in building models of secondary structures for nucleic acids and polynucleotides. However, such data only provide information about the stereochemistry of nucleosides and not the relative orientation of the nucleotides around the 3' and 5' phosphodiester bonds. This relative orientation is the key factor which determines the secondary structure of nucleic acids and details of this orientation can be obtained from single-crystal study of dinucleoside monophosphates and other higher oligomers. So far, crystal structure data on the dinucleoside monophosphates GpC (sodium salt)¹, GpC (calcium salt)², ApU³, UpA⁴, a dinucleotide pTpT⁵, a trinucleoside diphosphate ApApA⁶, and a tetranucleotide pApTpApT (M.A. Viswamitra, personal communication) are available. We have analysed the conformations of these molecules and present here stereochemical criteria for the backbone of the polynucleotide chains and the nucleic acids. Application of these criteria to nucleic acids indicates type II structure for DNA, as proposed previously by us⁷ as an alternative to the double helix.

Table 1 lists in the alphabetical notation the torsion angles of the sugar phosphate backbone of dinucleoside monophos-

Table 1 Backbone torsion angles for the fragments from dinucleoside monophosphates and related molecules

		$\zeta(5')$ C4'-C3'	α C3'-O3'	β O3'-P	γ P-O5'	δ O5'-C5'	ϵ C5'-C4'	$\zeta(3')$ C4'-C3'	Ref.
Set I	GpC	89	211	292	285	184	50	77	1
	GpC1	79	222	294	291	181	47	79	2
	GpC2	73	217	291	293	172	57	80	2
	GpC3	96	224	290	286	167	63	74	2
	GpC4	88	216	288	283	181	52	87	2
	ApU1	84	213	293	288	177	57	74	3
	ApU2	78	221	284	295	168	58	77	3
	ApA ⁺	82	223	283	297	160	53	81	6
	ApT*(1)	90	213	294	293	176	68	134	†
Set II	UpA(2)	77	224	164	271	192	54	93	4
	TpT*	157	252	163	288	187	41	158	5
	TpA*	134	204	168	286	186	49	83	†
Set III	UpA(1)	86	206	81	82	203	55	85	4
	A ⁺ pA ⁺	81	207	76	92	186	56	79	6
Set IV	A RNA	95	202	294	294	186	49	95	14
	A DNA	83	178	313	285	208	45	83	15
	B DNA	156	155	264	314	214	36	156	16
	C DNA	141	211	212	315	143	48	141	17
	D DNA	156	141	260	298	208	69	156	18
	B DNA (left)	140	200	212	282	165	40	140	‡

* Deoxy sugar.
† M. A. Viswamitra, personal communication.
‡ Present work.

phates and similar fragments in higher oligomers³; row 2 shows the respective bonds about which the torsion angles are measured. The sugar and nucleoside parts of these dinucleoside monophosphate fragments have conformational features similar to those of mononucleosides and nucleotides⁸. Thus

puckering of the sugar is either in the C3' *endo* or C2' *endo* region. The conformation about C5'-O5' is *trans* (*t*) and about C4'-C5' is *gauche-gauche* (*gg*). The major degrees of conformational freedom responsible for the orientation of the adjacent nucleotide units are about the phosphodiester bonds and accordingly these fragments have been grouped into three sets. Sets I, II and III have, respectively, the conformation *g⁻g⁻*, *tg⁻*, *g⁺g⁺* about the P-O bonds.

In sets I and II, γ varies from 271° to 302°, whereas β varies from 283° to 293° in set I and 163° to 168° in set II. In set III both β and γ are restricted from 76° to 92°. Thus for β , the observed conformations correspond to *g⁻*, *t*, *g⁺*, whereas for γ , they are *g⁻* and *g⁺*. The other torsion angle α varies from 204° to 224° except in TpT where it is 252°. Conformational energy calculations^{9,10} also indicate that *tg⁻*, *g⁺g⁺* conformations about the P-O bonds observed in the crystal structures are energetically as favourable as the *g⁻g⁻* conformation. The above conformational criteria of the backbone of the dinucleotide fragments have been used to predict possible secondary structures for polynucleotide chains.

Taking into account the conformational flexibility about the phosphodiester bonds and the possible variations in the remaining torsion angles, the conformational features of regular helical polynucleotide were analysed, using average bond lengths and bond angles for the monomer unit. Both right- and left-handed double helical structures with Watson-Crick base-pairing schemes were studied. The details of the calculations and the results will be reported elsewhere. Here, we discuss first the single chain helical parameters *n* and *h* (*n* is the number of nucleotide residues per turn and *h* the height per residue) which are relevant to the conformation of double stranded polynucleotides. As stated above, the major degrees of conformational freedom and the key to the conformation of nucleic acids are about the P-O3'(β) and P-O5'(γ) bonds. The salient features of the results are therefore presented in the β - γ space keeping other torsion angles fixed (Fig. 1). Although the crystal data given in Table 1 suggest only *gg* conformation about the C4'-C5' bond, we have also considered *gt* and *tg* conformations about this bond to determine the sensitivity of

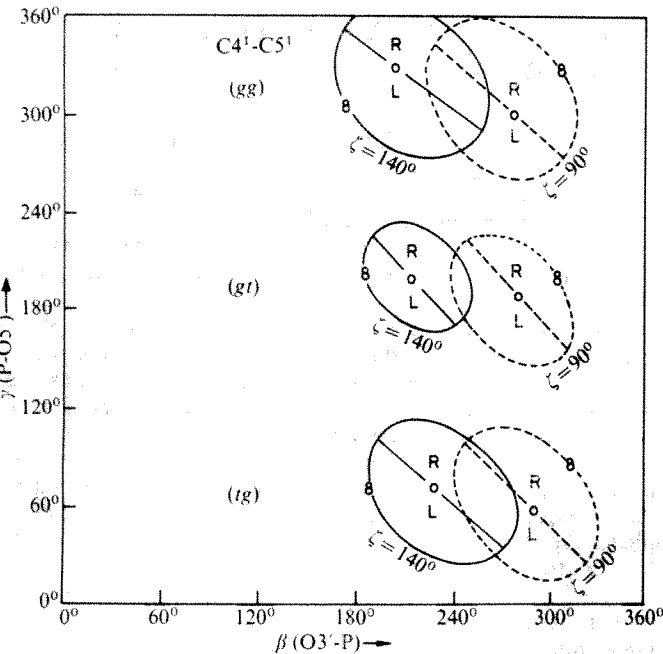


Fig. 1 Helical domains in the β - γ space corresponding to the conformations *gg*, *gt* and *tg* about the C4'-C5' bond, for C2' *endo* (solid line) and C3' *endo* (dashed line) sugar pucker. Only *n*=8 and *h*=0 are shown. Right and left helical sections are denoted by R and L.

the helical parameters with these conformations. The results are shown for two representative values of ζ , 90° and 140° ; the former in the C3' *endo* region and the latter in the C2' *endo* region. Interestingly the helix forming domains shown in Fig. 1 contain both right- and left-handed helices, and small changes in the values of β and γ alter the sense of the helix. Also the helix that forms domains in the β - γ space is sensitive to the sugar puckering and the conformation about the C4'-C5' bond. Thus a change in ζ from 90° to 140° produces a shift in β from g^- to t . Thus a change in the conformation about the C4'-C5' bond also produces a corresponding change in the conformation about γ . If the conformations about the C4'-C5' bond are gg , gt and tg , then in the helical domains the corresponding conformations about the P-O5' bond are g^- , t and g^+ respectively.

The observed conformation about the C4'-C5' bond is gg , so the helical domains in the β - γ space will be restricted to g^-g^- and tg^- . These are precisely the conformations about the P-O bonds that are observed in the crystals and given under sets I and II of Table 1. When g^+g^+ conformations of set III are used, no regular helical polynucleotide structures can be generated^{11,12}. But these conformations in the middle of a polynucleotide can reverse the chain direction¹³. In other words, g^+g^+ conformations about the P-O bonds can lead to a fold or bend along its backbone. Besides, in the case of a helical polynucleotide, the g^+g^+ conformation in the middle can change the handedness of the helix. We have used this conformation in the bend region of the type II model for DNA⁷.

Set IV in Table 1 lists the backbone torsion angles of right-handed double helical structures for nucleic acids reported in the literature¹⁴⁻¹⁸ for comparison with the crystal data. The agreement between A-RNA¹⁴ and the crystal data is very good. In contrast the agreement between the various conformations of DNA and the observed data is not good. In fact, the agreement is poor for both B and D DNA^{16,18}— α is much lower than the lowest observed value. Also, angles β and δ in B DNA and β in D DNA are different from the observed values.

To establish the conformational angles of the backbone for different forms of DNA which are close to the observed angles, helical domains were computed in the α - β space keeping γ , δ

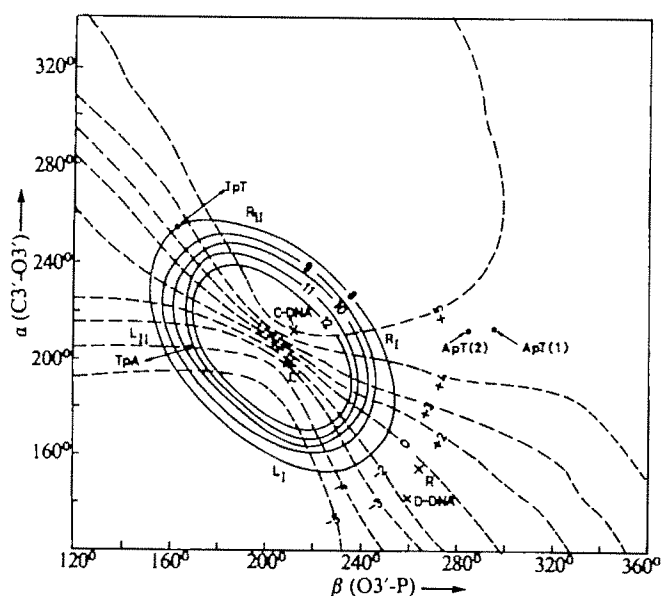


Fig. 2 Helical domains in the α - β space for C2' *endo* puckering ($\zeta = 140^\circ$). n (solid line) and h (dashed line) contours and observed torsion angles α and β of the fragments with C2' *endo* puckering for the sugar are shown. R and L denote right- and left-handed B DNA

and δ fixed in their respective average values (Table 1). The helical domains (also n and h contours) thus obtained for the average values of ζ in the C2' *endo* and C3' *endo* regions are given in Figs 2 and 3. The α , β values given under sets I and II are also plotted in these figures to show roughly the type of helical structures that will be generated for these fragments.

As before, the $h = 0$ contour divides the helical domains into right and left helical sections, and an iso- h contour cuts an iso- n contour at two places. Thus, as shown in the diagram, four regions, R_I and R_{II} in the right and L_I and L_{II} in the left helical sections are possible for single chains. For a given n and h value, in each helical domain two right and two left helical

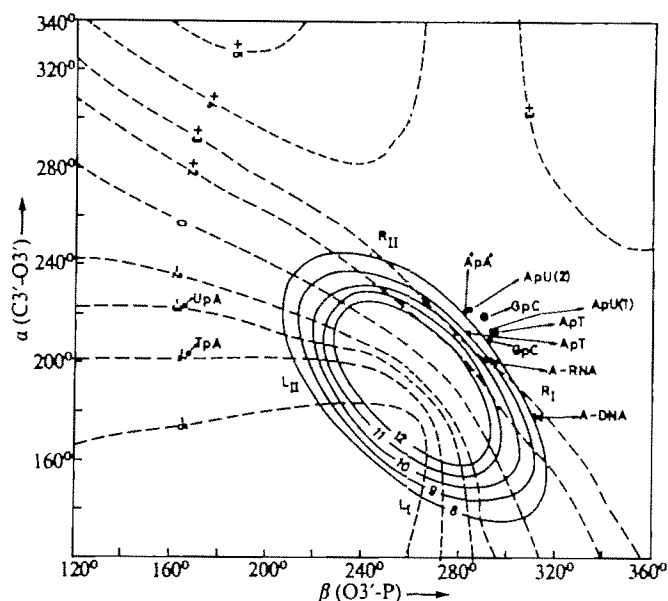


Fig. 3 Helical domains in the α - β space for C3' *endo* puckering ($\zeta = 90^\circ$). n (solid line) and h (dashed line) contours and observed torsion angles α and β of the fragments with C3' *endo* puckering for the sugar are shown. For GpC (Ca) the plotted conformation refers to the average values of the four independent molecules in the crystal.

structures are possible. However, not all of them lead to double helical structures with Watson-Crick base pairing.

In the C2' *endo* case shown in Fig. 2, the observed conformations ApT(1) and ApT(2) have very small n values and hence double helices are not possible. TpA and TpT lie in the L_{II} regions. In both these regions the bases are away from the helix axis and hence base-paired double helical structures are again not possible. Thus, double-helical structures are not generated when one uses the observed backbone conformational angles in the C2' *endo* region.

In the R_I and L_I regions, however, the bases overlap and hence double helical structures are possible. In fact by allowing the variations that are observed in the remaining torsion angles one can construct a series of stereochemically feasible right- and left-handed double helical structures consistent with the helical parameters of B DNA. The values of α and β for a left-handed double-helical structure with the B DNA parameters which we have constructed are plotted in Fig. 2 together with those for the right-handed helical structure¹⁶. We are now investigating whether X-ray fibre diffraction data could be used to distinguish between left-handed and right-handed models which have conformational angles as close as

possible to those observed in the crystals. The only remaining conformational angle χ about the glycosidic bond has a value near 0° for the left-handed structure while $\chi \approx 75^\circ$ for the right-handed structure, as reported previously⁷.

In Fig. 3, for the C3' *endo* case, except UpA and TpA, the other observed conformations are clustered in R₁ with $n = 8$ to 11 and $h = 2$ to 3 Å. Thus double helical structures can indeed be generated with the observed backbone conformational angles in the C3' *endo* region. In the case of both L_I and L_{II} regions, the bases overlap, so double helical structures are possible.

Thus for the *gg* conformation about the C4'-C5' bond, the helical domains occur in the β - γ space at the g^-g^- and tg^- regions, which are precisely the observed regions of conformations for the fragments given. Small variations in dihedral angles bring about changes in the sense of the helix and both right- and left-handed double helical structures are possible. It is clear that conformations similar to the fragments in sets I, II and III can lead to right helical, left helical and bend (or fold) structures respectively. A structural model for B DNA can thus be constructed with right and left helical segments having backbone conformations similar to sets I and II respectively. Conformations similar to set III serve to link consecutive right and left segments of a chain. A structure thus arrived at is a type II structure proposed previously by us⁷ as an alternative structure for DNA. The type II structure is not a double helix but consists of a pair of polynucleotide strands held together by Watson-Crick base pairing. Each of the two strands has alternating right- and left-handed segments approximately five base pairs long. As a consequence of mixing right-handed with left-handed helical segments and vice versa, each strand has bends or folds. These bends impart a significant amount of flexibility to the polynucleotide. This inherent flexibility of the type II structure can permit the initiation of the supercoiled structure of DNA following energetically allowed folds. This flexibility along the backbone could provide a possible solution to the compression of DNA within chromatin¹⁹.

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Corrigendum

In the article 'Decision making in animals' by D. J. McFarland, *Nature* **269**, 15 (1977), the sentence starting in line 16 on page 18 should read: On this basis we can express the expected probability of fertilisation, given that the male is still involved in the courtship as

$$\varepsilon \int_0^T [u_1(t) + e^{\varphi S(t)} u_3(t)] S(t) x(t) dt.$$

Also on page 18, the scheme based on Dempster's formulation (ref. 14) should read as shown below:

Objective: Maximise likelihood of successful fertilisation

$$\int_0^\infty \{ \exp [-\varphi S(t)] S(t) u_1(t) x(t) + S(t) u_3(t) x(t) \} dt$$

Plant equation: $\dot{x}(t) = -x(t)^{-\rho} u_2(t) + \theta$

Work during courtship: $\frac{u_1(t)}{\delta} + \frac{u_2(t)}{\gamma} + \frac{u_3(t)}{\tau} \leq 1$

Energy expended during courtship:

$$\int_0^\infty \left[\frac{u_1(t)}{\delta} + \frac{u_2(t)}{\gamma} + \frac{u_3(t)}{\tau} \right] dt \leq E$$

Hamiltonian:

$$H = e^{-\varphi S(t)} S(t) u_1(t) x(t) + S(t) u_3(t) x(t) - F(t) \left[-x(t)^{-\rho} u_2(t) + \theta \right]$$

(Costate) Hamiltonian equation:

$$\dot{F}(t) = \frac{\partial H}{\partial x} = e^{-\varphi S(t)} S(t) u_2(t) - \rho \frac{F(t)}{x(t)^{1+\rho}} u_2(t) + S(t) u_3(t)$$

states: S = spermatophore count (0-3)

x = oxygen 'debt'

controls: u_1 = rate of display behaviour (maximum rate δ)

u_2 = rate of creep behaviour (maximum rate γ)

u_3 = rate of spermatophore transfer (maximum rate τ)

parameters: φ = basic discount rate of display behaviour
 ρ = control parameter for rate of decrease of x through skin respiration during creep behaviour

θ = rate of increase of oxygen debt with time (during display and transfer behaviour)

E = maximal energy expended in courtship

F = costate variable = marginal value of likelihood of success per unit of x

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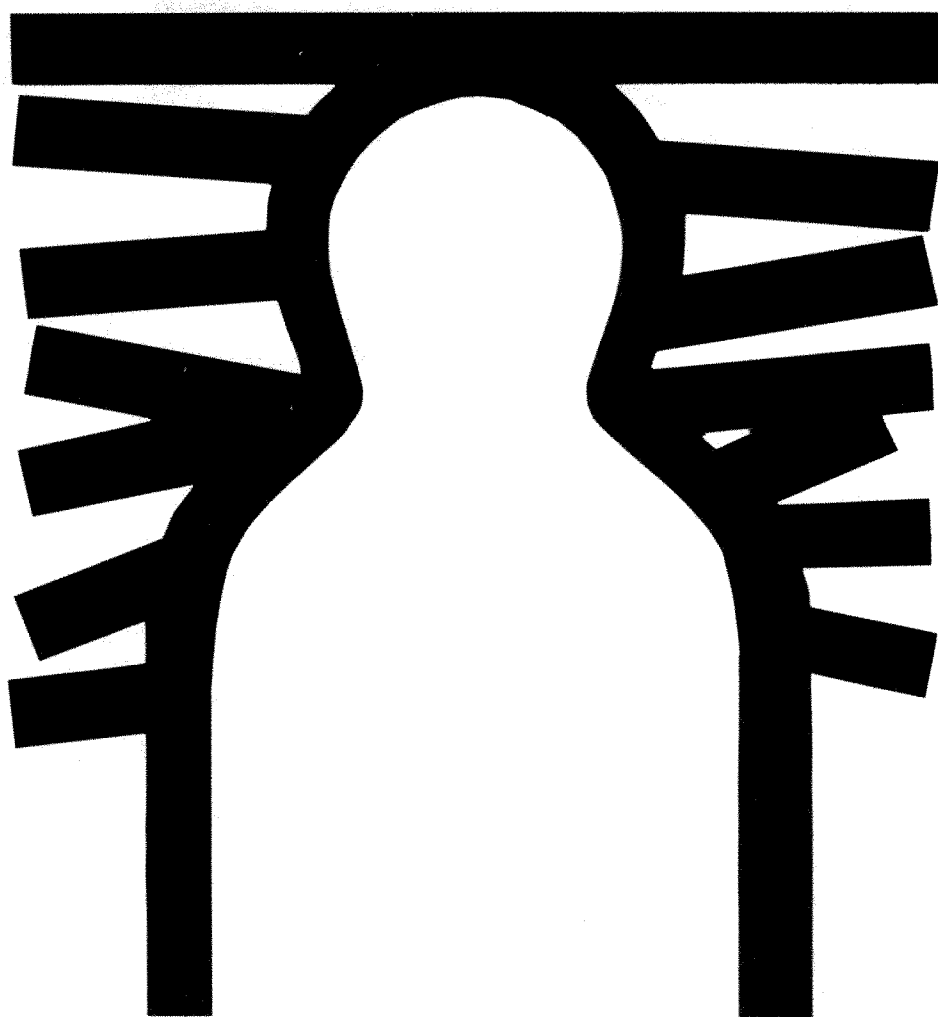
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reviews

Coming to terms with Nature

Eric Ashby

The Twenty-Ninth Day: Accommodating Human Needs and Numbers to the Earth's Resources. By L. R. Brown. Pp. 363. (Norton: New York, 1978.) \$11.95.

THE title of this book is another example of the hypnotic effect of exponential and sigmoid curves upon those who have never used them in serious scholarly work. The lily pond which doubles its leaf-area every day and is half-full on the twenty-ninth day will, so the introduction to this book says, be full on the thirtieth day. Had the author taken the trouble to read the brilliant analysis of population growth published by Alfred Lotka over half a century ago in *Elements of Physical Biology*, he might have hesitated before starting his argument with such a misleading example. The dilemma facing mankind is not what to do about exponential growth; it is how to devise political systems which can be used to manage the second-order consequences which follow the point of inflection of a sigmoid curve of growth.

Forecasts of population growth, oil reserves, capacity of the land to produce food, resources of minerals: all these vary by whole orders of magnitude. Disputes about the forecasts miss the point: that all these parameters already are, or sooner or later will be, above the point of inflection of a sigmoid curve; and the general characteristic of this part of the curve is that feedback, which was positive while the curve was convex to the time-axis, now becomes negative.

Discontinuities invariably occur: nations like China successfully limit the birth rate; nuclear power may replace oil for some purposes; plant breeders may increase yield per hectare, aluminium may replace copper. Some of the discontinuities (for example, the substitution of steam-power for water- and wind-power) have profound social consequences, which have been well documented. But, discontinuities apart, the trend is indisputable; exponentials become sigmoid and the seminal social problem is not how to delay the point of inflection by technological innovation, nor is it how to bring forward the point of inflection by rationing; it is how to manage society when many

measurable variables lie on the upper reaches of sigmoid curves.

This is the worthy theme in Lester Brown's book. In the first nine chapters he covers familiar, not to say overcrowded, ground: the evidence for over-fishing, over-grazing, deforestation; the inevitable rise in population, the geopolitics of energy production, the economic stresses which followed a sixfold increase in oil prices imposed by OPEC and (though western countries are more reticent about this) a two-and-a-half-fold increase in wheat prices imposed by the grain-producing nations and a threefold increase in the price of newsprint; and the growing gap between affluence and poverty.

His sources of information for the material in these chapters are practically all from journalistic and sometimes emotive accounts in the press or in popular books. His exposition of these issues is no worse, and no better, than that in a dozen other books covering similar ground. So it is to the last three chapters, which are about how society is to come to terms with Nature, that I turned with some anticipation. For Lester Brown, unlike many middle-class élitists keen on conservation, has really encountered the world-dilemma at close quarters, as an agricultural officer, as a dweller in Indian villages, and with the Overseas Development Council. This experience (I hoped) would dissuade him from advocating the utopian remedies which prompt decision-makers to drop such books into the waste paper basket.

That Lester Brown's three chapters on accommodation to the new phase of world economy do not provide encouraging solutions is not his fault. No one has come up with even the rudiments of a solution. But Lester Brown does put the emphasis where it belongs, namely on the need to find solutions for social and ethical problems rather than material problems. The great disillusion is not directed against the technologists; it is directed against the economists; and he quotes a telling remark made by Pierre Trudeau: "Inflation has not found its Keynes. I personally think the Keynes of inflation will not be an economist . . ." but will instead "be a political, philosophical or moral leader inspiring people to do without the excess consumption so

prominent in the developed countries". The difficulty is that this recipe may be valid for the inhabitants of Dorking and Wimbledon (well heeled suburbs of London) but not for the swarms of people who squat on the streets of Calcutta. Up to a point there is a close correlation between growth in GNP and the overall improvement in quality of life; it is in affluent societies that the correlation breaks down. So—and this is the conclusion that hurts—the initiative lies in Dorking and Wimbledon and not in Calcutta. And the choice before Dorking and Wimbledon is voluntary adjustment to austerities or submission to bureaucratic regulation.

Some writers—Heilbroner, for example, and Ophuls—are pessimistic about the outcome of this choice. They see (as Heilbroner puts it) the latitude for voluntary choice diminishing "under the slowly closing vise of environmental constraint". Lester Brown is more optimistic, though I confess he does not support his optimism with encouraging arguments. All the same, he is right to be optimistic. The ultimate treason to mankind is to lose confidence in humanity. □

Lord Ashby is a Fellow of Clare College, Cambridge and Chancellor of the Queen's University, Belfast, Northern Ireland.

Photoemission from surfaces

Photoemission and the Electronic Properties of Surfaces. Edited by B. Feuerbacher, B. Fitton and R. F. Willis. Pp 540. (Wiley: Chichester and New York, 1978) £19.50.

THE study of photoemission from solids has grown enormously in the past decade. Although the photoelectric effect has been known for many years and provided a key result for the development of quantum theory, its application to the study of electronic structure of solids is much more recent and the appreciation of its essential surface sensitivity has encouraged its inclusion in the growing armoury of techniques of surface science. Indeed, in the case of ultraviolet photoelectron

spectroscopy in particular, the degree and nature of its surface sensitivity is still an issue of considerable interest. The extent of this development is mirrored by the size of this book, a collection of 17 separate articles on the theory and experimental results of the technique, together with two articles on the intimately related problem of the electronic structure of clean surfaces and adsorbates on surfaces.

The editors have collected together a formidable team of authors for this work; very few of the 'big names' in the subject are missing and the resulting articles are authoritative and thorough. Of course, in any multi-author book, particularly with authors active in an expanding and developing research field, the treatment of the subject is not always even and overlaps do exist, particularly in the introduction of the basic ideas behind several articles. Frequently, however, this overlap is beneficial; the reader is presented with contrasting views of the same material and can emerge with a more balanced appreciation as a result.

Biology of RNAs

The Ribonucleic Acids. (Second edition.) Edited by P. R. Stewart and D. S. Letham. Pp. 374. (Springer: New York, Heidelberg and Berlin, 1978.) DM47.40; \$23.70.

As an antidote to the new-style undergraduate textbooks, awash with coloured diagrams and divided into easily digested segments, *The Ribonucleic Acids* is recommended to students. Here the content is solid, the illustrations mainly restricted to structural formulae in sombre black and white.

This book is intended for students who already have basic training in biochemistry, and, although dealing with a specialist subject, does not aim to be either fully comprehensive or overly detailed. The first chapter is, typically, an historical introduction by the editors and is followed by chapters dealing with different species of RNA—nuclear, transfer, messenger, and so on—and their transcription and translation. The final chapter is a collection of recipes for the preparation and fractionation of RNA, curious in a textbook designed for students but undoubtedly of value to research workers.

Although the chapters have been written by different authors, some attempt has been made to make the style and structure uniform. Reading of the interaction between the 3' end

An intrinsic problem of any book in a very active research field is one of timing; has the subject developed sufficiently to warrant a book or will it be rapidly dated by new developments? In this case the timing seems to be a good compromise. Certainly new developments are occurring, and will continue to occur, which may change the detailed interpretation of specific problems. Also, the construction of many new synchrotron radiation sources around the world is bound to cause a shift in emphasis to the increased use of photon energy and polarisation-dependent effects as a means of studying surfaces. On the other hand, the stress in many articles in this volume is on basic physical principles and as such the book is likely to prove valuable for several years to those involved in, or wishing to learn more about, photoemission from surfaces and their electronic structure.

D. P. Woodruff

D. P. Woodruff is Lecturer in Physics, University of Warwick, UK.

of 16S ribosomal RNA and the 5' end of mRNA in four different chapters suggests that a certain uniformity in content was also required! The balance between cataloguing the facts and critical appraisal of experimental results varies in different chapters, although perhaps some RNAs—nuclear RNA, for example—elicit a more controversial literature.

Distinguishing the characteristics of prokaryotes and eukaryotes is very much easier for research workers, where it is a case of 'us and them', than it is for students, who have no previous affiliations. The latter group of readers will find this book adds to their problems. Sometimes prokaryotes and eukaryotes are allocated different headings, sometimes they are contrasted in the same sentence. This makes the text easier to read and less repetitious, but will undoubtedly perpetuate the existence of that interesting ribosome, found only in undergraduate essays, having 30S and 50S subunits containing 18S and 28S RNAs.

The references accompanying each chapter are extensive, reviews being listed separately. This information, together with the up-to-date overview of the biology of RNA that the text provides, should make this a useful book for research workers in related fields as well as the students for whom the book was written.

Pamela Hamlyn

Pamela Hamlyn is at the MRC Laboratory of Molecular Biology, Cambridge, UK.

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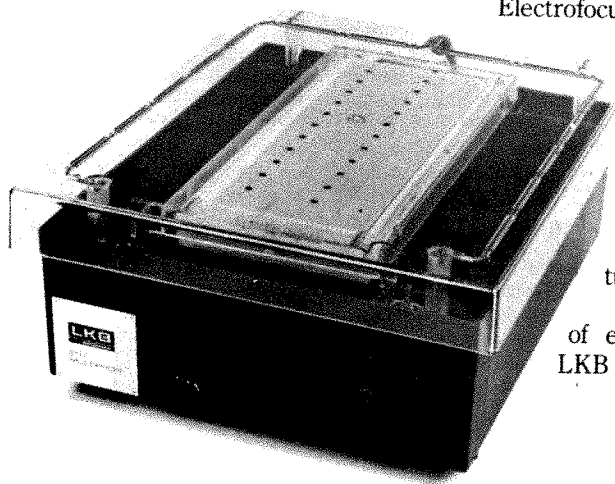
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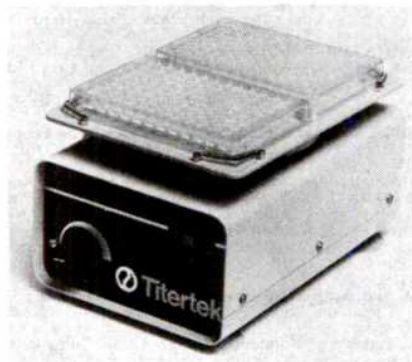
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- ROBINSON, Colin, and MORGAN, Jon. *North Sea Oil in the Future: Economic Analysis and Government Policy*. Pp.xvii + 216. ISBN-0-333-23772-2. (London and Basingstoke: The Macmillan Press, Ltd., 1978. Published for the Trade Policy Research Centre, London.) £10 net.
- ROCKS OF THE WORLD. Flat pack. 36 specimens. In the Beginning. Pp.16. ISBN-0-08-022921-2. (Oxford: Pergamon Press, 1978.) Flat pack £15; Library pack £25.
- SMITH, Bruce D. *Prehistoric Patterns of Human Behaviour: a Case Study in the Mississippi Valley*. With a contribution by Wilma Wierstrom. (Studies in Archaeology.) Pp.xvii + 264. ISBN-0-12-650650-7. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) \$19; £12.35.
- SPEAKING OF SCIENCE 1977. (Volume 30 of the Proceedings of The Royal Institution of Great Britain. A Collection of Evening Lectures.) Pp.294. ISBN-0-85066-135-8. (London: Taylor and Francis, Ltd., 1978.) £8.
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- THE GAS INDUSTRY AND THE ENVIRONMENT. (Proceedings of a Symposium of the Committee on Gas, Economic Commission for Europe, Minsk, Byelorussian SSR, 20-27 June 1977.) Pp.xvi + 263. ISBN-0-08-022412-1. (Oxford and New York: Pergamon Press, 1978. Published for the United Nations.) \$34.
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- VERSCHUUR, Gerrit L. *Cosmic Catastrophes*. Pp.x + 214. ISBN-0-201-08099-0. (Reading, Mass. and London: Addison-Wesley Publishing Company, 1978.) Hardcover £7.50; Paperback £3.75.
- WAGNER, Richard H. *Environment and Man*. Third edition. Pp.xiii + 591. ISBN-0-393-09066-3. (New York: W. W. Norton and Company, Inc., 1978.) \$12.95.
- WHALE MANUAL 78. Pp.iv + 153. ISBN-0-905966-05-8. (London: Friends of the Earth, 1978.) £2.
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- YOUNG, Stanley P. *The Bobcat of North America: Its History, Life Habits, Economic Status and Control*. With List of Currently Recognized Subspecies. Pp.193 + 29 plates. ISBN-0-8032-5894-1. (A Wildlife Management Institute Publication.) (Lincoln and London: University of Nebraska Press, 1978.) \$3.50; £2.45.
- YOUNG, Stanley, P., and JACKSON, Hartley H. T. *The Clever Coyote*. Part 1: Its History, Life Habits, Economic Status, and Control. Part 2: Classification of the Races of the Coyote. Pp.xv + 411 (81 plates) ISBN-0-8032-5893-3. (A Wildlife Management Institute Publication.) (Lincoln and London: University of Nebraska Press, 1978.) \$5.50; £3.85.
- ZAHAVY, Zev. *Whence and Wherefore: The Cosmological Destiny of Man Scientifically and Philosophically Considered. An Analysis Relating to "In the Centre of Immensities" by Sir Bernard Lovell*. Pp.178. ISBN-0-498-02190-4. (South Brunswick and New York: A.S. Barnes & Co. London: Thomas Yoseloff Ltd., 1978.) \$7.50.

newly on the market

Microtitration plate shakers. Flow. Fixed and variable speed Titertek microtitration plate shakers are now available from Flow Laboratories. Both models use an orbital mode of vibration which has been shown to give even mixing in all plate wells in the minimum time consistent with cell stability and lack of splashing. They have been laboratory tested to prove their ability to resuspend RBCs which have been allowed to sediment overnight. The advantages offered over the manual methods of resuspending are uniformity of results and time/labour-saving.

Circle No. 39 on Reader Enquiry Card.



The Flow Titertek

Nitrate electrode. Electronic Instruments. A nitrate electrode has been added to the range of ion-selective electrodes of Electronic Instruments. Model 8006-2 is of the capsule design ensuring easy assembly and minimal maintenance. The new ion exchanger used gives improved selectivity for NO_3^- in the presence of Cl^- , an important breakthrough in nitrate determinations in sewage and industrial effluents.

Circle No. 40 on Reader Enquiry Card.

Electrophoresis power supplies. Buchler. Buchler Instruments has introduced two new DC power supplies designed for immuno-, paper and thin layer electrophoresis techniques. Each unit features easy to read, oversized meters and a single control for varying the output. One unit has ranges of 0–350 VDC, 0–100 mA and the other unit provides 0–1,000 VDC, 0–200 mA. Both of these units have dual output jacks so that more than one chamber may be operated at the same time.

Circle No. 41 on Reader Enquiry Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Form.

Disposable transfer pipette. Variable Volumetrics. Variable Volumetrics introduce a disposable transfer pipette with ultra lean tube. This polyethylene transfer pipette has a tube so narrow that it will fit into almost any size test tube. It is a one-piece pipette complete with its own bulb, and will eliminate the danger of broken glass, operator contamination from having to change rubber bulbs and cross contamination of samples with contaminated pipettes or rubber bulbs. The pipette delivers 21–24 drops per ml, and has a maximum capacity of 3 ml.

Circle No. 42 on Reader Enquiry Card.

Screening test for blood lead. MSE. An instrument is now available with which a blood sample can be checked in about 10 s to give an indication of the presence of lead in the body. The instrument, the ESA model 4000, uses fluorimetry to measure the amount of zinc protoporphyrin in a drop of blood (about 30 μl). Using the instrument, hundreds of blood samples can be checked in a day. The model 4000 is portable, and extremely easy to operate. The presence of lead in the environment during the analysis cannot affect the result.

Circle No. 43 on Reader Enquiry Card.

UV-monitors and recorders. Pharmacia. Pharmacia presents its new range of UV-monitors and recorders for detecting proteins, polypeptides and nucleic acids in liquid chromatography. The dual path Monitor UV-2 incorporates a flow-cell which allows the simultaneous measurement of the absorbance of a flowing liquid at two different path-lengths. The two measuring systems are completely independent: each can be operated to measure optical density or % transmission at 254 nm or 280 nm. The 1 mm system allows measurements to be made with excellent linearity in eight ranges up to 20 A at the same time as the 20 mm system detects solute concentrations in eight ranges down to a full scale deflection only 0.005 A. The Single Path Monitor UV-1 offers the choice of monitoring absorbance in eight ranges up to 2 absorbance units per cent transmission at 254 nm or 280 nm with a 3 mm or 10 mm path-length flow cell. Also, the one-channel recorder REC-1 and two-channel recorder REC-2 provide a 250 nm recording width, 16 chart speeds and 11 calibrated input ranges with an accuracy of $\pm 0.3\%$ full scale deflection.

Circle No. 44 on Reader Enquiry Card.

Triaxial accelerometer. RIL. The model 507T triaxial accelerometer is a new addition to the BBN range of pre-amplified accelerometers marketed by RIL. It is ideal for applications where low frequencies must be detected or monitored. Technical characteristics include: a flat 10 mV per g response over a frequency range of 0.3–7,000 Hz for each axis; an exceptionally low noise floor of 20 μV broadband, amplitude linearity is $\pm 1\%$ to $+21\text{ g}$ peak; and mounted frequency response is 26 kHz. The preamplifiers in each accelerometer are safeguarded from self-generated transients up to 500 V by a unique protection circuit.

Circle No. 45 on Reader Enquiry Card.

ECG amplifier. British American Optical. A cardio-monitor, designated Model 1134, has been announced. The equipment combines an ECG amplifier and heart rate meter with alarms, in a single compact unit. Patient safety is ensured by the use of isolation transformers, leakage is $< 10\mu\text{A}$ at chassis connectors and insulation withstands 8 KV patient to chassis potential. Remote ECG recording may be initiated manually by means of a push-button. Required power supply is 230 V, 50 Hz.

Circle No. 46 on Reader Enquiry Card.



The British American Optical Model 1134

Protease detection kit. Bio-Rad. Bio-Rad's protease detection kit provides a convenient way to measure the activity of serum proteases, or of other proteases such as trypsin, α -chymotrypsin and pronase. When dissolved, tablets from the kit form an agar gel containing a protein preparation which gives it a turbid appearance. The sample to be assayed is placed in a well and diffuses into the gel forming a clear ring where the protein is digested. Proteolytic activity can then be determined very simply from the ring diameter, using guides provided in the kit.

Circle No. 47 on Reader Enquiry Card.

announcements

Awards

The following awards have been made by the Royal Society: the Copley Medal to **Professor R. B. Woodward** (Harvard University) for his contributions to experimental and theoretical organic chemistry; the Rumford Medal to **Sir George Porter** (Royal Institution) for his work on flash photolysis; the Davy Medal to **Professor A. Eschenmoser** (Swiss Federal Institute of Technology) for his work on synthetic organic chemistry; the Darwin Medal to **Dr G. Pontecorvo** (Imperial Cancer Research Fund) for his advances in genetics utilising lower organisms; the Hughes Medal to **Professor W. Cochran** (University of Edinburgh) for his advances in crystallography and lattice dynamics; the Leverhulme Medal to **Sir Frederick Warner** (Cremer and Warner) for his contributions to the developments of chemical and allied processes on the industrial scale; The Royal Society Eso Award to **A. G. Frame, J. M. Lalthwalte, A. D. Evans, Dr J. F. W. Bishop and Dr T. N. Marham** for their contributions to the development of the prototype fast reactor at Dounreay; the Royal Society Mullard award to **Dr J. W. Black** for his work on the development and discovery of two new types of drugs.

The 1978 ICE Diagnostics Techniques prize winners are **L. M. Callow and K. Hladky** of UMIST for their work on an electrochemical impedance corrosion monitor. The prize of £1,000 is to be awarded annually for five years and is to be administered by the Institution of Chemical Engineers. Entries for the 1979 award are now invited. ICE, 165-171 Railway Terrace, Rugby.

The following have been awarded the Indian National Science Academy's Medals for Young Scientists for the year 1978: **Dr Praveen Chaddah; Dr Renu Khanna Chopra; Dr K. C. Das; Dr A. R. Datta; Dr Vinay V. Deodhar; Shri S. Easwaramoorthy; Dr Chunilal Ghosh; Dr Jitendra Nath Goswami; Dr S. Ramasesha; Dr H. A. Ranganath; Dr Kalidas Sen.**

The Institution of Nuclear Engineers has awarded the first Hinton Medal to **Anthony R. W. Lunt** for his part in the engineering of the Dounreay prototype fast reactor.

The Beilby Medal and Prize for 1978 has been awarded by the RIC, the SCI and the Metals Society to **Dr John Christopher Scully** for his work in corrosion science in particular on stress-corrosion cracking. The award is made to British investigators (normally under the age of 40) for original work in chemical engineering, fuel technology or metallurgy. Applications for the 1979 award should be made to: The Sir George Beilby Memorial Fund, The RIC, 30 Russell Square, London WC1.

The trustees of the Lady Tata Memorial Trust for research in leukaemia have made the following international awards for the academic year 1978-79: from Great Britain—**H. Bayani; A. Karpas and H. Jacobs**; from France—**J. Grosslerner**; from Portugal—**E. Cardoso**; from Spain—**R. de N. Bastos**.

The Harrison Memorial Prize will be awarded to a British subject under the age of 30 years who has conducted the most promising original investigations in chemistry and published the results in a scientific periodical. Applications to the President of the Chemical Society, Burlington House, Piccadilly, London W1, by 31 December 1978.

The sixth of the Henry R. Worthington Technical Award for a prize of 10,000 dollars held under the auspices of the EEC is to be launched on a European-wide basis. It is to be awarded to young researchers from the universities and industry, working in the field of applied mechanics for the movement of fluids and for energy conversion systems. The contest regulations are available from Award Secretariat, Via Pirelli, 19-201234 Milan, Italy.

Meetings

2-3 October, **Workshop on Placenta—the Neglected Experimental Animal**, London (Dr Peter Beaconsfield, SCIP Research Unit, Bedford College, University of London NW1, UK).

2-4 October, **Symposium on Comparative Pathology of Zoo Animals**, Washington (Dr Montali, National Zoological Park, Washington, D.C. 20008).

2-5 October, **Clean Air Ways and Means**, Brighton (National Society for Clean Air, 163 North St, Brighton, Sussex, UK).

3-6 October, **Wind Energy Systems**, Amsterdam (2nd ISWES, BHRA Fluid Engineering, Cranfield, Bedford, UK).

3-6 October, **Postsynthetic Alterations of Information Molecules**, Lunteren (Miss Elly Looy, Institute of Molecular Biology, Padualaan 8, Utrecht, The Netherlands).

3-7 October, **Nuclex 78**, Basle (Congress Service of the Swiss Industries Fair, PO Box CH-4021 Basle, Switzerland).

4-6 October, **National Conference of Standards Laboratories**, Gaithersburg (Brian Belanger, A345 Physics Building, NBS, Washington, D.C. 20234).

4-7 October, **3rd European Symposium on Hormones and Cell Regulation**, Bischenberg (H. J. van der Molen, Dept of Biochemistry II, Medical Faculty, Erasmus University, PO Box 1738, Rotterdam, The Netherlands).

5-6 October, **Carcinogenicity of Foods**, Chicago (Dr John J. Hefferren, ADA Health Foundation Research Institute, 211 East Chicago Ave, Chicago, Illinois 60611).

5-6 October, **Future Trends in Clinical and Laboratory Research in Cancer**, Paris (Dr W. H. Fridman, IRSC, BP No. 8, 94800 Villejuif, France).

5-6 October **International Liquid Chromatography Symposium: Biological/Biomedical Applications**, Boston (Gerald L. Hawk, International Division, Waters Associates, Inc. Milford, Massachusetts 01757).

6-1 October, **Annual Meeting of the Swiss Society of Mineralogy and Petrography**, Brig (Prof. M. Grunfelder, Institut de Cristallographie et Petrographie ETH, 8092 Zurich, Switzerland).

9-12 October, **4th International Congress for the Study of Bauxites, Almina and Aluminium**, Athens (Prof. S. S. Augusthis, National Technical University, Dept of Mineralogy, Petrology and Geography, PO Box 1006, Athens, Greece).

9-13 October, **1st International Congress on Bentonites**, Sassari (Istituto di Mineralogia e Geologia, Facoltà di Agraria, Università de Sassari, Italy).

9-19 October, **Atmospheric Sensing with Lasers**, London (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

10-12 October, **3rd Annual Conference on Materials for Coal Conversion and Utilization**, Gaithersburg (Bernard Levin, B64, Technology Building, NBS, Washington, D.C. 20234).

10 October, **Satellite Doppler Tracking and Geodetic Applications**, London (The Royal Society, 6 Carlton House Terrace, London SW1, UK).

10-13 October, **4th European Electro-optics Conference and Exhibition**, Utrecht (Carole Meads, Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

10-14 October, **International Symposium on Interaction of Marine Geodesy and Ocean Dynamics**, Miami (Dr George A. Maul, NOAA-AOML, 15 Rickenbacker Causeway, Miami, Florida 33149).

11 October, **Carcinogenic Risks from Food—Real or Imaginary?** London (SCI Food Group, 14 Belgrave Square, London SW1, UK).

12-14 October, **International Symposium on Pituitary Microadenomas**, Milan (Secretariat, Clinica Neurochirurgica dell'Università, Ospedale Policlinico, Pad. Beretta, via F. Sforza 35, 20122 Milan, Italy).

12-18 October, **1st Ibero American Congress of Cell Biology**, Mendoza (Secretariat, Casilla de Correo 56,5500 Mendoza, Argentina).

12-18 October, **4th Latin American Congress of Electron Microscopy**, Mendoza (Secretariat, Casilla de Correo 56,5500, Mendoza, Argentina).

Reports & Publications

UK & Ireland—August

Directorate of Ancient Monuments and Historic Buildings. The Scientific Treatment of Material from Rescue Excavations. (A Report by a Working Party of the Committee for Rescue Archaeology of the Ancient Monuments Board of England.) Pp.28. (London: Directorate of Ancient Monuments and Historic Buildings, DOE, Fortress House, Savile Row, 1978.) [18]

Centre for Agricultural Strategy. Strategy for the UK Dairy Industry. (CAS Report 4.) Pp.186. (Reading: Centre for Agricultural Strategy, The University, 1978.) £2.95. [18]

Bulletin of the Geological Survey of Great Britain. No. 66: The Pleistocene Succession at Kenn, Somerset. By D. D. Gilbertson and A. B. Hawkins. Pp. iv+41. (London: HMSO, 1978.) £3.75 net. [18]

Central Electricity Generating Board. Renewable Sources of Energy: The Prospects for Electricity. By Glyn England. Pp. 11. (London: CEBG, Publicity Office, 15 Newgate Street, EC1, 1978.) [38]

Scottish Plant Breeding Station. Fifty-seventh Annual Report, 1977/1978. Pp. 148. (Pentlandsfield, Roslin, Midlothian: Scottish Society for Research in Plant Breeding, 1978.) [38]

The British Industrial Biological Research Association. Annual Report 1977. Pp. 55. (Carshalton, Surrey: The British Industrial Biological Research Association, 1978.) [38]

Project Daedalus: The Final Report on the British Interplanetary Society Starship Study. Edited by Dr. A. R. Martin. (Journal of the British Interplanetary Society Supplement.) Pp. 192. (London: British Interplanetary Society, 12 Bessborough Gardens, SW1, 1978.) £6; \$12 post free.) [48]

Department of Industry. Research and Development Requirements and Programmes Report 1977-78. Pp. 44. (London: Department of Industry, Technology Library, Abell House, John Islip Street, SW1, 1978.) [48]

The Importance of Being Curious. By Professor Hans L. Kornberg, FRS. (The Third Leverhulme Memorial Lecture.) Pp. 26. (Liverpool: Liverpool University Press, 1978.) [118]

Departments of the Environment and Transport. Report on Research and Development 1977. Pp. 66. (London: HMSO, 1978.) £2.25 net. [118]

Journal of Petroleum Geology, Vol. 1, No. 1, July 1978. Pp. 1-122. Published quarterly. Subscriptions: £20 for one year, UK only, post-free, by surface mail; \$US40 to all other countries, post-free, surface mail. (Beaconsfield, Bucks: Scientific Press, Ltd., 1978.) [148]

National Institute of Agricultural Botany. Fifty-Eighth Report and Accounts, 1977. Pp. 112. (Cambridge: National Institute of Agricultural Botany, Huntingdon Road, 1978.) [148]

Progress in Perspective: a View of Bayer. By René Cutforth. Pp. 48. (Richmond, Surrey: Bayer UK, Ltd., Bayer House, 1978.) [168]

Department of Energy. Digest of United Kingdom Energy Statistics 1978. Pp. vii+138. (A Publication of the Government Statistical Service.) (London: HMSO, 1978.) £7.50 net. [168]

Seawatching. By Tony Soper and Noel Cusa. Pp. 32. (Over, Cambridge: Dinosaur Publications, Ltd., 1978. Published for the National Trust.) 60p. [188]

Smith Kline and French Foundation: Smith Kline Foundation. Annual Report 1977.9. (Welwyn Garden City, Herts.: Smith Kline and French Foundation, 1978.) [188]

Threshold Limit Values for 1977. (Guidance Note EH 15/77 from the Health and Safety Executive) Pp. 20. (London: HMSO, 1978) 30p. [188]

John Innes Institute. Sixty Eighth Annual Report, 1977. Pp. 183. (Norwich: John Innes Institute, 1978.) £2.50. [218]

Office of Population Censuses and Surveys. Mortality Statistics: Accidents and Violence. (Review of the Registrar General on Deaths Attributed to Accidental and Violent Causes in England and Wales, 1976.) Pp. viii+39. (London: HMSO, 1978.) £1.50 net. [258]

Where Were You, Brother?: An Account of Trade Union Imperialism. By Don Thomson and Rodney Larson. Pp. 138. (London: War on Want, 467 Caledonian Road, N7, 1978.) £1.20. [298]

Council for National Academic Awards. Annual Report for 1977. Pp. 75. (London: Council for National Academic Awards, 344/354 Gray's Inn Road, WC1, 1978.) [298]

Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 289. No. 1365: The Origin of the Triassic Clay Assemblages of Europe with special reference to the Keuper Marl and Rhaetic of Parts of England. By C. V. Jeans. Pp. 549-639+plates 1-5, pullout 1. UK £13.25; Overseas £13.75. Vol. 290. No. 1366: Sea Floor Development—Moving Into Deep Water. A Discussion organized by Sir Angus Paton, F.R.S., Sir Peter Kent, F.R.S., Sir George Deacon, F.R.S., Sir Kenneth Hutchison, F.R.S., and M. B. F. Ranken, in collaboration with the British National Committee on Ocean Engineering of the Council of Engineering Institutions. Pp. 1-189+plates 1-7; two pullouts. (London: The Royal Society, 1978.) [318]

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Other countries—August

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APPOINTMENTS VACANT

AGRICULTURAL RESEARCH COUNCIL

UNIT OF NITROGEN FIXATION

University of Sussex
Brighton BN1 9QJ

MOLECULAR BIOLOGIST

Is required to join an interdisciplinary team working on the physiology, biochemistry and genetics of nitrogen fixation.

The vacancy is for a specialist in molecular biology who will collaborate with genetics and biochemists in a collective study, *in vitro* and *in vivo*, of the products and regulations of the nitrogen fixation genes of *Klebsiella*.

Candidates should have a first or upper second class Honours degree in biochemistry, microbiology or microbial genetics and at least two years' relevant postgraduate research experience.

Appointments as Higher Scientific Officer on the salary scale £4,101 to £5,448 with non-contributory superannuation. Starting salary according to experience.

Applications to the Administrator, Unit of Nitrogen Fixation at the above address. Closing date September 29, 1978. 7099(A)

UNIVERSITY OF BIRMINGHAM

DEPARTMENT OF GEOLOGICAL SCIENCES

RESEARCH ASSOCIATE IN HYDROGEOLOGY

Applications are invited for the above post to carry out hydrogeological interpretation related to chalk aquifer water resources studies. The study will include hydrochemical appraisal and some borehole and surface geophysics. The work is supported by a research grant from the Anglian Water Authority. Applicants should have a good honours degree in geology and at least an M.Sc. degree in hydrogeology. The post is for three years from November 1, 1978. The successful applicant may be permitted to register for the degree of Ph.D.

Salary in the range £3,384 to £4,882, plus superannuation, maximum starting salary £3,883.

Applications should be sent to Assistant Registrar, Science and Engineering, University of Birmingham, P.O. Box 363, Birmingham B15 2TT (three copies) including curriculum vitae and the names of three referees by Wednesday, September 27, 1978. Please quote Reference NG8. 7135(A)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE (University of London)

Keppel Street, WC1E 7HT

IMMUNOLOGY UNIT

A RESEARCH TECHNICIAN

is required for a period of up to two years to work on the mechanisms of immune complex disease under the direction of Dr M. W. Steward. Experience in biochemical and immunological techniques is essential.

Salary, depending on age, experience, etc., will be in the range £3,615 to £4,284 inclusive.

Applications, consisting of full education and career details and naming two referees, should be sent to Secretary (A1) at the School. 7136(A)

UNIVERSITY OF WARWICK

RESEARCH ASSOCIATE

in the

DEPARTMENT OF CHEMISTRY AND MOLECULAR SCIENCES

Applications are invited for the post of RESEARCH ASSOCIATE with Dr B. E. P. Swoboda to study the detailed mode of action of the anti-tumour agent, cyclophosphamide on normal and cancer cells. Some experience with cell culture, organic syntheses and/or radioisotope techniques would be advantageous and the applicant should have a good degree in Biochemistry or a related subject.

The appointment, which is supported by the Cancer Research Campaign, is initially for one year, with the possibility of extension to three years. Maximum starting salary will be £3,660 per annum on Research Range 1B, plus superannuation. Applications (two copies) giving qualifications and experience and naming two referees should be sent to the Academic Registrar, University of Warwick, Coventry CV4 7AL, as soon as possible, quoting Ref No 4/2A/78. 609(A)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF GENETICS

CYTOGENETICIST

Applications are invited for the above post. The chief requirements include familiarity with modern methods of clinical diagnosis of cytogenetic abnormality, including banding methods and tissue culture. The laboratory is well equipped and there are opportunities for research.

The starting salary will be between £5,000 and £6,000 within Range 1A Research and Analogous scale, according to age and experience.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by September 30, 1978. 7051(A)

CSIRO AUSTRALIA

Postdoctoral Research Fellow

Division of Forest Research Yarralumla, A.C.T.

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7000 employees—2400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Soil Compaction

General: Modern harvesting machines may cause considerable disturbance to the forest floor, including soil compaction. The Division is concerned about the impact of such disturbance, in native as well as plantation forests, on the soils, hydrology and growth during second rotation cropping.

Duties: Applications are invited from persons interested in initiating research on aspects of soil compaction. The successful candidate will receive cooperation from the Division's sections concerned with soil physics, hydrology and harvesting, but is expected to develop an independent program.

Qualifications: Ph.D. or equivalent qualifications in soil physics or soil mechanics and demonstrable research ability. A broad understanding of forest operations and catchment hydrology is desirable.

Salary: Research Scientist or Senior Research Scientist: \$A14,829 — \$A21,643 p.a.

Tenure: Fixed term of 5 years, with Australian Government Superannuation benefits.

Applications IN DUPLICATE, stating FULL personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 1069/119 should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON WC2R 3EH by 15th October 1978.

Applications in U.S.A. and Canada should be sent to: The Counsellor (Scientific), Embassy of Australia, 1801 Massachusetts Avenue, N.W., WASHINGTON, D.C., 20036, U.S.A.

7090(A)

Laser Scientists

RUTHERFORD LABORATORY

South Oxon.

The Rutherford Laboratory is the Science Research Council's principal establishment for advanced work on the frontiers of physics, and one of Europe's leading centres for laser research.

The Laser Division now has vacancies for two Scientific Officers in experimental physics.

Plasma Physics

The successful applicant will join a small experimental team providing facilities for and collaborating with University users of the SRC Laser Facility. The experiments are concerned with the study of laser-plasma interactions and laser driven implosion and compression. The work involves the alignment of small targets in the focus of the laser beam, operation of plasma measurement devices, analysis of experimental data and development of new measurement systems.

You should have a University degree in a relevant subject. Experience in optics or pulse electronics would be advantageous.

Target Fabrication and Development

The selected candidate will become part of a small group preparing microscopic but complex targets for irradiation by the high power laser beams.

You will carry out development work on new target manufacture measurement and coating techniques and be responsible for the supply of targets for the experimental programme. The work involves the use of vacuum and high pressure gas handling equipment, optical microscopes and interferometers, X-radiography equipment and a scanning electron microscope.

You should have an HNC in Applied Physics or equivalent qualification in a relevant subject. Experience in thin film coating techniques or use of any of the above equipment would be desirable.

Scientific Officer salaries are in the £3,037 – £4,724 (including supplements) with prospects of promotion to higher grades and salary scales.

Working conditions are excellent and benefits include generous holidays, sick leave allowance and contributory superannuation scheme.

Write or telephone for an application form to:
Ruth Jeans of the Personnel Group.
Please quote reference VN735 and state
which post you are interested in.
Closing date for applications 30th Sept. 1978

RUTHERFORD
Science Research Council

Rutherford Laboratory, Chilton, Didcot,
Oxfordshire OX11 0QX. Tel Abingdon 21900

7132(A)

RESEARCH ASSISTANT

Graduate required to join a team of scientists investigating basic aspects of cardiac biochemistry and physiology. A training in physiology or biochemistry and experience in spectrophotometry, tissue metabolite analysis or organ perfusion would be advantageous. The research unit is located in the new Rayne Institute and appointments will be made through St Thomas's Hospital Medical School. Applications together with a curriculum vitae should be sent to Dr D. J. Hearse, The Rayne Institute, St Thomas's Hospital, London SE1.

7031(A)

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THE UNIVERSITY OF PAPUA NEW GUINEA (PORT MORESBY)

Applications are invited for the post
of

TUTOR/ ASSISTANT LECTURER

in the
DEPARTMENT OF GEOGRAPHY

Preference will be given to candidates with teaching and research interests in Agricultural Geography and/or Human Ecology. The appointee will be required to make a major contribution to the Social Studies course in the Arts Faculty Foundation Year, and the Man and His Environment course in the Science Faculty. In addition he/she will have teaching duties in some of the more Advanced Geography courses. There are ample opportunities for research in Papua New Guinea and if appropriate the appointee will be encouraged to enrol for a higher degree. Appointment will be for a period of two years in the first instance. Salary scale: K7,323 to K8,382 p.a. (£1 sterling=K1.33). In addition, an allowance of K1,300 p.a. if single is payable. An extra K1,000 Marriage Allowance and K156 per child Child Allowance may also be payable. There is also provision of housing and annual leave fares. Detailed applications (2 copies) with curriculum vitae and naming three referees plus small photograph to be sent direct to Secretary, Box 4820, University PO, Papua New Guinea by October 6, 1978. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

7093(A)

INSTITUTE OF PSYCHIATRY TECHNICIAN

required in the Department of Neuropathology to assist with electronic data processing connected with biochemical research. An applicant keen to learn need have only some of the relevant biological or mathematical background. Training will be given in data processing and in electronic and biochemical techniques, according to the applicant's background and previous experience. Unusual opportunity to gain broader experience of modern microprocessor-based techniques.

This position is grant supported for three years with possibility of extensions. Whitley Council conditions of service. Salary in the range £3,261 to £4,680 per annum plus London Weighting £354 per annum.

For application form please write to the Assistant Secretary, Institute of Psychiatry, De Crespigny Park, Denmark Hill, London SE5 8AF or telephone 703 5411, Ext. 214, quoting reference OEP/N.

7092(A)

LAKEHEAD UNIVERSITY POSTDOCTORAL FELLOW SURFACE CHEMISTRY

Position available in small group active in surface chemistry and heterogeneous catalysis. Stipend and benefits up to maximum allowable by N.R.C. regulations.

Please forward applications with names of two referees to:

Dr R. A. Ross
Professor of Chemistry
Lakehead University
Thunder Bay
Ontario
Canada P7B 5E1

7122(A)

N.M.R. SPECTROMETRY ASSISTANT

King's College, London

Applications are invited for the
above

TECHNICIAN GRADE 5

post to assist in the operation/maintenance of a Bruker HFX 90 F¹ n.m.r. spectrometer. Previous experience of spectroscopic or of other advanced electronic instrumentation desirable. Initial instruction will be given if required. Salary on scale £3,651 p.a. rising to £4,185 p.a. (inclusive) for a 37½ hour week Monday to Friday. 4 weeks' annual holiday. Contributory pension scheme. Applications in writing with full details to: The Head Clerk, Ref. 204008, King's College London, Strand, WC2R 2LS

7071(A)

UNIVERSITY OF OXFORD DEPARTMENT OF AGRICULTURAL SCIENCE RESEARCH ASSISTANT

with a good honours degree, a driving licence and an interest in fungal taxonomy and pathology to assist on a N.E.R.C. funded project to monitor disease in plant communities over three years.

Starting salary £3,189 on Grade 1B.

Applications with the names of two referees by September 30 to the Administrator, Department of Agricultural Science, Parks Road, Oxford OX1 3PF, from whom further particulars can be obtained.

7123(A)

MAX-PLANCK-INSTITUTE Tübingen, Germany POSTDOCTORAL RESEARCH POSITION IN TUMOUR VIROLOGY

open, beginning January 1979, or earlier. Applicants with Ph.D. or M.D. degrees should have some experience in immunology and/or (tumour) virology to join a research group working on cell surface and nuclear alterations of virus transformed cells.

Salary around 30,000 German Mark per annum.

Some knowledge of German is desirable, but not absolutely necessary. Applications should be sent to:

Dr R. Kurth
Friedrich-Miescher-Laboratorium
Max-Planck-Institutes
Spemannstr. 37-39
74 Tübingen, W. Germany

7124(A)

MOREDUN RESEARCH INSTITUTE BIOCHEMISTRY DEPARTMENT S.O./H.S.O.

A SCIENTIST is required to join a small team. The appointee will be responsible for initiating and conducting research on the biochemical consequences of parasitism and certain other diseases in ruminants. Applicants should have a first or upper second class Hons. Degree in Biochemistry or Agricultural Chemistry. At least 2 years postgraduate experience is required for appointment as H.S.O.

Salary scale: S.O. £2,839 to £4,415. H.S.O. £4,101 to £5,448. Non-contributory superannuation scheme. Starting salary will be dependent upon qualifications and experience.

Applications giving the names and addresses of 2 referees should be sent to the Secretary, Animal Diseases Research Association, Moredun Institute, 408 Gilmerton Road, Edinburgh EH17 7JH.

7125(A)

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UNIVERSITY
OF MANCHESTER
DEPARTMENT OF MEDICAL
BIOCHEMISTRY
POSTDOCTORAL
RESEARCH ASSISTANT
IN BIOCHEMISTRY

A postdoctoral research assistant sponsored by the Medical Research Council is required from October 1978, earlier if preferred. The work, under the supervision of Jacqueline B. Weiss, involves the characterisation and spatial recognition of new collagen alpha chains and the person appointed will be part of a multi disciplinary team of whom one is an electron microscopist. Experience in collagen biology or biochemistry would be a very welcome but experience in mineral protein chemistry would also be acceptable. The appointment is for three years only. The salary scale is in the range £4,365 to £4,834 p.a. Applications, including curriculum vitae, to Jacqueline B. Weiss, Senior Lecturer, Department of Medical Biochemistry, Medical School, Stopford Building, Oxford Road, Manchester M13 9PT. 7075(A)

THAMES POLYTECHNIC
SCHOOL OF MATERIALS
SCIENCE AND PHYSICS
RESEARCH ASSISTANTS

A research assistant is required in the Division of Physics to investigate the properties of semiconductor layers grown by liquid phase epitaxy. A second research assistant is required in the Division of Materials Science to investigate, either the properties and preparation of (a) new organic luminescent materials, or (b) new photo-voltaic materials.

Candidates should have good honours degrees in physics, materials science or chemistry. Previous experience in the above areas is not required and successful candidates will register for higher degrees of the C.N.A.A.

Salary scale, £3,015 to £3,189 inclusive.

Further particulars and form of application may be obtained from the Staffing Officer, Thames Polytechnic, Wellington Street, London, SE18 6PF, to whom completed applications should be returned by September 26, 1978. 7080(A)

ROTHAMSTED
EXPERIMENTAL STATION
Harpenden, Herts. AL5 2JQ
STATISTICIAN

to be responsible for a continuing agricultural survey. The work involves liaison with collaborating organisations, organisation of data coding, analysis and reporting of results. There will also be opportunities for research on problems of interpreting observational data.

Qualifications: Degree, HNC or equivalent qualification in Statistics with at least five years' relevant post-qualifying experience. Previous experience with responsibility for staff would be an advantage.

Appointment in grade of Higher Scientific Officer (£4,101 to £5,448 p.a.). Starting salary according to experience. Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees and quoting Ref 369 by October 5, 1978. Further details on request. 7070(A)

UNIVERSITY OF
EDINBURGH
DEPARTMENT OF BOTANY
POSTDOCTORAL
RESEARCH FELLOW

A Postdoctoral Research Fellow is required for two years for an experimental investigation of "The Transition to Flowering in *Silene*". This work is supported by the Agricultural Research Council and will involve the experimental manipulation and measurement of the growth of plants in controlled environments, measurement of cell cycle parameters by microdensitometry and autoradiography, scanning electron microscopy, and histology.

Salary on range 1A, £3,660 to £6,178 per annum, according to age and experience.

Applications, with a curriculum vitae and the names and addresses of two referees, should be sent as soon as possible to Dr R. F. Lyndon, Department of Botany, University of Edinburgh, Mayfield Road, Edinburgh EH9 3JH. Please quote Reference 5048. 7119(A)

ST GEORGE'S HOSPITAL
MEDICAL SCHOOL
(University of London)
RESEARCH ASSISTANT

Graduate or postdoctoral person required in the Physiology Department to participate in a 2 to 3 years Arthritis and Rheumatism Council research project into the control of fluid movement between the synovial cavity and micro-circulation of mammalian joints. Applicants should hold an upper-second or first-class degree in animal physiology and have a special interest in mammalian cardiovascular/body fluid physiology. Excellent research facilities are available in this modern, recently established Department and the opportunity exists for a graduate to undertake a higher degree during the tenure of the post. Salary scale £3,212 to £3,447 p.a. plus £450 London allowance. Applications, with the names of two referees, to the Establishment Officer, St George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. 7104(A)

THE
UNIVERSITY OF LEEDS
DEPARTMENT OF
PLANT SCIENCES

Applications are invited for the temporary fixed-term post of post-doctoral RESEARCH FELLOW to work with Dr L. D. Incoll on a project concerned with work on when and how cytokinins open graminaceous stomata.

Ideally applicants should have been trained at the postgraduate level in research on phytohormones and should have research experience in that field. Appointment is for one year in the first instance, renewable for up to a total of three years. Initial salary will be in the range £3,883 to £4,382 per annum (within the 1A range of the scale for Research and Analogous staff.) (Under review).

Applications, giving curriculum vitae and the names of two referees, should be sent by October 5, 1978 to Dr L. D. Incoll, Department of Plant Sciences (Bailey Wing), The University of Leeds, Leeds LS2 9JT, from whom further details may be obtained. 7073(A)

Coal Survey Specialists

£7000-£8825 based Yorkshire

The National Coal Board have opportunities for 2 Coal Quality Scientists to work as part of a National Exploration team based in Yorkshire which has the task of establishing the long term coal reserves of the nation.

The main work will involve the synthesis of data from boreholes as exploration proceeds; the identification of those quality parameters to which the profitability of any coal mining venture would be most sensitive; and the evaluation of each prospect within both its Regional and National exploration setting. The successful applicants will also participate in appraising the coal quality and utilisation aspects of the Board's major capital investments programme for the development of new capacity.

Applicants should hold an honours degree in either chemistry, geochemistry, fuel technology or a related subject. Experience in one or more of the following fields would be an advantage: fuel technology, coal analysis and utilisation, coal preparation, geological data analysis.

A period of supervision and training will be given where appropriate.

These are permanent positions within Headquarters Geological Branch with an inclusive salary range of £7000-£8825. Attractive conditions of employment.

Please apply with full details to:

NCB

Headquarters Staff Manager,
National Coal Board,
Coal House, Doncaster, South Yorkshire.

7134(A)

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**THE NEW SOUTH WALES
INSTITUTE OF TECHNOLOGY**
Sydney, Australia

SCHOOL OF LIFE SCIENCES
**Associate Head of School and Head,
Department of Cellular Pathology
and Microbiology**

The New South Wales Institute of Technology is a corporate tertiary institution, established to provide a wide range of professional courses for those entering or already employed in industry, government, and technological fields.

The School occupies a six-storey building at Gore Hill adjacent to the Royal North Shore Hospital. There are well equipped laboratories for teaching and research in Pathology, Microbiology, Biochemistry, Environmental Biology, Physical Biology and General Biology. The School also jointly operates the Gore Hill Research Laboratories with the Royal North Shore Hospital.

The appointee will be responsible to the Head of School for the teaching and development of undergraduate and postgraduate programmes in cellular pathology and microbiology. This will include the continuing development of courses in immunology, haematology, diagnostic cytology and clinical microbiology. The appointee will also be required to study current trends in the fields mentioned, and to make recommendations on new undergraduate and postgraduate courses.

It is anticipated that the appointee will be qualified in medicine or an area of medical science, possess appropriate postgraduate qualifications and have substantial professional experience. Depending on their interests and experience they may be offered an association with the Royal North Shore Hospital.

Salary will be in the range of \$A29,612 — \$A31,789. With consent of Council, academic staff are permitted to undertake limited consulting work.

The position offers tenure, superannuation, long service leave, and a Housing Loan Scheme. Fares and a contribution toward removal and initial accommodation expenses are provided for overseas appointees.

Dr R. L. Werner, President of The Institute, will be in London from the 11th to the 26th of September, and will be available to provide further information on this position through the Agent General's office on 01-839 6651.

Applications close on October 31, 1978. Applicants should arrange for three confidential referees' reports to arrive by the same date. Applications should include: address; phone number; personal particulars; documentary evidence of qualifications; work and teaching experience; affiliations; publications; research work undertaken and the names and addresses of the referees contacted. Applications and referees' reports are to be sent to:

The Agent-General for N.S.W.
N.S.W. Government Offices
66 STRAND
LONDON WC2N 5LZ
ENGLAND

7091(A)

**THE POLYTECHNIC
OF NORTH LONDON**
PHYSICS DEPARTMENT

Applications are invited from graduates for a

RESEARCH ASSISTANTSHIP
in experimental physics. The fields of research are:

Development of microwave excited spectroscopic light sources;
Investigation of the properties of thin magnetic films;

Development of low background counters for studying cosmic and environmental radiation.

Salary scale: £3,015 to £3,189 (including London Allowance). Duties include six hours of teaching per week. A suitably qualified candidate may be allowed to register for a research degree.

Applications should be made as soon as possible to the Head of the Department of Physics, Polytechnic of North London, Holloway, London N7 8DB, from whom further details may also be obtained.

7094(A)

NEUROSCIENCES FACULTY

Faculty positions available in the Neurosciences Program, Maryland Psychiatric Research Center, affiliated with the University of Maryland School of Medicine. One position is open to a

SCIENTIST

with rigorous postgraduate training in neuroendocrinology, basic expertise in hormone assay procedures and the capability to study functional aspects of hormones/polypeptides in a multi-disciplinary clinical research centre. The second position is for a SCIENTIST with rigorous postgraduate training in a neurobiology specialty with preference given to strength in biochemistry and/or genetics plus experience in cell or organ culture techniques.

Submit curriculum vitae, summary of research interest and two letters of reference by November 15, 1978, to: Neurosciences Search Committee, Maryland Psychiatric Research Center, Box 3235, Baltimore, Md. 21228. An Equal Opportunity Employer.

7060(A)

THE UNIVERSITY OF CALGARY

**MORPHOLOGICAL
SCIENCE**

The Faculty of Medicine, The University of Calgary, Calgary, Alberta, invites applications for the position of Professor and Head, Division of Morphological Science.

Candidates should have an established record of achievement as a scholar, an active research programme, and a strong background related to teaching anatomy in a medical school using the systems method.

Applications, including an up-to-date curriculum vitae should be forwarded to:

Dr. L. E. McLeod, Dean
Faculty of Medicine
The University of Calgary
Calgary, Alberta
Canada
T2N 1N4



7128(A)

**PUBLIC HEALTH
LABORATORY
SERVICE BOARD
SCIENTIFIC OR SENIOR
SCIENTIFIC OFFICER
AT THE MYCOLOGICAL
REFERENCE LABORATORY,
LONDON SCHOOL OF
HYGIENE AND
TROPICAL MEDICINE,
Kippell Street (Gower Street),
London WC1E 7HT**

Applications are invited from graduates who have a primary training in medical mycology or microbiology and experience with the handling and identification of pathogenic micro-organisms. The successful candidate will be required to assist with the handling and identification of pathogenic fungi with serodiagnostic and antifungal tests, and to develop independent research interests in the field of medical mycology. The level of appointment will depend on qualifications and experience but will be on the scale £2,991 to £4,899 (Scientific Officer) or £5,451 to £6,837 (Senior Scientific Officer), plus London Weighting allowance of £364. Terms and conditions of service similar to National Health Service. The laboratory may be visited by arrangement with the Director, Professor D. W. R. Mackenzie (telephone 01-636 8686 ext 361) who can provide a brief job description of the post. Applications to Professor Mackenzie at the London School of Hygiene and Tropical Medicine, stating date of birth, qualifications, experience including published work, and naming 3 referees. Closing date September 30, 1978. 7087(A)

TECHNICIAN (Grade 4) required to work in Fermentation Pilot Plant Laboratory. Duties include microbiological/analytical work and operation of laboratory fermenters. The successful applicant will be required to operate a limited day shift system (09.00 to 17.00 hrs: 6 x duty days, 3 x rest days). Salary in the range £3,674 to £4,121 p.a. inclusive of London Weighting and shift inconvenience allowance. Enquiries in writing to: Departmental Superintendent, Biochemistry Department, Imperial College, South Kensington, London SW7 2AZ. 7126(A)



**Southampton
THE
UNIVERSITY**

CHAIR OF GEOLOGY

Applications are invited for a further Chair of Geology which is to be established during the academic year 1978/79.

Salary within the professional range.

Applicants may have teaching and research interests in any field of study appropriate to a University Department of Geology.

Further particulars may be obtained from the Secretary and Registrar, The University, Southampton SO9 5NH, and applications (10 copies from applicants in the United Kingdom) must be submitted before November 5, 1978. 7130(A)

CHANGE OF ADDRESS

The Advertisement offices for NATURE have moved to
3 DYERS BUILDINGS, LONDON, EC1N 2NR
TELEPHONE : 01 831-6901

**ROYAL FREE HOSPITAL
SCHOOL OF MEDICINE
(University of London)**

BIOCHEMICAL GENETICIST

Research Assistant wanted to work on inherited variations of proteins in mice. Starting salary £3,834 p.a. (scale 1b). Applications, with names of two referees, to be sent as soon as possible and not later than September 29, to Professor R. J. Perry, Royal Free Hospital School of Medicine, 8 Hunter Street, London WC1N 1BP. 7100(A)

**UNIVERSITY OF OXFORD
DYSON PERRINS LABORATORY
(ORGANIC CHEMISTRY)**

Applications are invited for a post-doctoral research assistant supported by the Medical Research Council to work in the mechanism of action of cyclic macrolide antibiotics. Experience in nuclear magnetic resonance is desirable but not essential, and this will be the main technique used. The appointment will be for a period of up to two years in Research Support Grade 1A at an appropriate point of the salary scale up to £4,365 p.a. plus U.S.S., the preferred starting date is January 1, 1979. Applicants should write as soon as possible giving full personal details and naming two referees to Dr J. M. Brown, Dyson Perrins Laboratory, South Parks Road, Oxford OX1 3QY. 7102(A)

**UNIVERSITY OF LEICESTER
DEPARTMENT OF GENETICS**

TWO Post-doctoral positions are available to work on the mechanism of action of penicillin and the use of the antibiotic to study the processes of bacterial cell division and shape determination.

ONE position is M.R.C. funded for three years and the other is funded by Iocchst Pharmaceuticals for two years in the first instance. Both positions are on the salary scale 1A (£3,660 by £235, under review) and are available from October 1 or as soon as possible hereafter.

Applications including a curriculum vitae and the names of two referees should be sent to Dr Brian G. Spratt, Department of Genetics, University of Leicester, Leicester LE1 7RH as soon as possible. 7101(A)

**Geology and Geophysics
UNIVERSITY OF
CALIFORNIA, BERKELEY**

An Assistant Professorship in Geology is an anticipated vacancy, pending budgetary approval, to be filled July 1, 1979 in the Department of Geology and Geophysics. Candidates must have an expertise which will contribute to an integrated programme of field and laboratory research and teaching. Research emphasis may be in the areas of metamorphic petrology, structure-stratigraphy, or tectonics. The position will require teaching and research supervision in the field. The Ph.D. degree is required.

Applications should be submitted by December 15, 1978 to:

T. V. McEvilly, Chairman
Geology and Geophysics
University of California
Berkeley, Ca 94720

and should include the names of three academic referees.

The University of California is an Affirmative Action employer. 7105(A)

**AGRICULTURAL
RESEARCH COUNCIL
INSTITUTE OF
ANIMAL PHYSIOLOGY
Babraham, Cambridge CB2 4AT
VASCULAR BIOLOGY**

Applications are invited for a post in the Department of Cell Biology, tenable for three years, on a project financed by the M.R.C. This project, under the supervision of Dr J. L. Gordon, will involve studies on the control of vascular tone and permeability, using cultured endothelial and smooth muscle cells, and isolated vascular preparations. Experience with such techniques, with biochemical correlates of contractility, or with transport, metabolism or secretion of vasoactive agents (e.g. prostaglandins and amines) would be advantageous. Candidates should possess a 1st or upper 2nd class Honours degree in biochemistry or related science, together with at least 2 years' relevant post-qualifying experience. Preference will be given to candidates with relevant postdoctoral experience. Appointment will be to the H.S.O. grade (£4,101 to £5,448) or to the S.S.O. grade (£5,154 to £6,898) depending on experience. Applications, including C.V. and the names of two referees, should be sent to the Secretary By September 30, 1978. Quote Ref J.L.G. 7106(A)

**AUSTRALIAN NATIONAL
UNIVERSITY**

Applications are invited for appointment to the following:

**Research School of
Biological Sciences
DEPARTMENT OF
POPULATION BIOLOGY
RESEARCH FELLOW**

The Department has a small group interested in genome structure and evolution in eucaryotes. Current interests encompass both genetic as well as molecular studies on *Drosophila* and rodent genomes, with emphasis on highly repeated DNA. The applicant should have postdoctoral research experience and be interested in developing a programme on some aspect of genome structure. The applicant should preferably be familiar with current molecular techniques, preferably DNA cloning, restriction endonuclease digestion of DNA and electron microscopy.

Appointment of Research Fellow is for three years in the first instance with the possibility of extension to five years.

CLOSING DATE October 15, 1978
SALARY Salary on appointment will be in accordance with qualifications and experience within the range Research Fellow \$A15,179 to \$19,811. Current exchange rates \$A1=\$US1 15 58p.

OTHER CONDITIONS Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with housing is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain the further particulars from the Association of Commonwealth Universities (Acpts), 36 Gordon Square, London WC1H 0PF. 7097(A)

**CSIRO
AUSTRALIA
Post Doctoral
Research
Fellow
Division of
Plant Industry
Canberra Act**

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7000 employees—2400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Molecular Biology

General: The Division, with headquarters in Canberra, ACT, conducts pure and applied research in genetics, plant breeding, genetic resources, biochemistry, physiology, microbiology, plant nutrition, ecology, crop adaptation, soil fertility and plant introduction.

The position is in the Plant Storage Protein Group which is an interdisciplinary unit incorporating biochemists, geneticists, physiologists and plant nutritionists. Work in progress includes studies on the biosynthesis of pea storage proteins and on the characterization of the genetic elements involved. Related activities in the Division include studies on chromosome structure and replication, the genetics of regulatory processes, and a study of the organisation, function and expression of the chloroplast genome.

Duties: To participate in a research program involving molecular studies on gene expression in higher plants. It is anticipated that recombinant DNA procedures will be used, in conjunction with other molecular biology techniques, to analyse in detail the organization and expression of storage protein genes in peas.

Qualifications: Applicants should have a Ph.D. degree in an appropriate field or postgraduate research experience of equivalent standard or duration supported by satisfactory evidence of research ability.

Salary: The appointment will be made within the salary ranges of Research Scientist or Senior Research Scientist: \$A14,829 — \$A21,643 p.a.

Tenure: The position is available for two years.

Applications **IN DUPLICATE**, stating **FULL** personal and professional details, the names and addresses of at least two professional referees, and **QUOTING REFERENCE NUMBER 130/1431** should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON WC2H 3EH by 15th October 1978.

Applications in U.S.A. and Canada should be sent to:—The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C., 20036, U.S.A.

7089 (A)

CHANGE OF ADDRESS

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TELEPHONE: 01 831-6901

Microbiologist

G. D. Searle is a leading Pharmaceutical company with its main U.K. site and Research laboratories at High Wycombe in Buckinghamshire.

Within the laboratories we are involved in a growing number of long-term research and development programmes in various fields of medical science.

The position of Microbiologist would ideally suit an experienced man or woman who would join the Research and Development Division to take on responsibility for several branches of microbiological work, primarily the monitoring of sterile production and testing facilities, but also providing microbiological advice for programmes on fermentation development and microbiological research programmes. The person appointed will report to the Biological Quality Control Manager.

Candidates should have several years' experience in this field, probably with a PhD and possibly with specific experience of microbiological aspects of tissue culture based vaccine production. All applicants must have at least a working knowledge of GMP requirements and should enjoy a real challenge - with the potential satisfaction of making significant advances in the world of medicine.

In addition to a competitive salary, conditions of employment include 4 weeks' holiday per year, a contributory pension fund, subsidised private health insurance, sports and social club and staff cafeteria.

Applications are requested, quoting reference M/R16, either in writing, giving career details, or by telephoning for an application form to: G. Jenkins, Personnel Manager, Research & Development, G. D. Searle & Co. Ltd., Lane End Road, High Wycombe, Bucks. (Tel. High Wycombe 21124 ext. 3272).



SEARLE

7129(A)

NATAL MUSEUM Pietermaritzburg, South Africa VACANCY FOR A DIPTERIST

Applications are invited for a vacancy in the Department of Entomology at the Natal Museum. The appointee will be required to take charge of the Department and undertake taxonomic research on Diptera. A person interested in one of the families Tachinidae, Cecidomyiidae, Dolichopodidae or Phoridae, is particularly sought, but applicants with other interests will also be considered. The minimum academic requirement is an Honours degree. Full details of the post and application forms can be obtained from the Director at 237 Loop Street, Pietermaritzburg 3201, South Africa. 7033(A)

MEDICAL RESEARCH COUNCIL TOXICOLOGY UNIT PHARMACOLOGIST/ PHYSIOLOGIST

required as part of a long-term programme to build up expertise for studies of the mechanism of action of toxic substances on specific organs or preparations *in vivo* and *in vitro*. He will have opportunities for interdisciplinary studies with other members of the staff as well as detailed discussions with Professor E. Zaimis of the Department of Pharmacology, Royal Free Hospital Medical School. The post will be a three-year post-doctoral temporary appointment.

Apply in writing by the end of September enclosing a full curriculum vitae to Dr W. N. Aldridge, Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF. 7048(A)

DIRECTOR DIVISION OF FORENSIC MEDICINE HEALTH COMMISSION OF NEW SOUTH WALES and PROFESSOR or ASSOCIATE PROFESSOR DEPARTMENT OF PATHOLOGY THE UNIVERSITY OF SYDNEY (Joint appointment—Health Commission and University of Sydney)

Salary: \$A35,275 p.a.

Qualifications: Medical practitioners eligible for registration in New South Wales and qualified and experienced in forensic pathology.

Duties: (1) Responsible to the Health Commission for the administration of the Division of Forensic Medicine which performs forensic autopsies on behalf of the Sydney City Coroner and the Coroners of several suburban areas and provides a forensic pathology consultant service to country areas in New South Wales, as well as forensic tests and examinations on behalf of the Police Department; and (2) responsible to the University of Sydney for teaching and research in Forensic Medicine in the Faculty of Medicine.

General: The successful applicant will be offered permanent appointment to the New South Wales Health Commission under the New South Wales Public Service Act (1902). Subject to medical examination, he/she will be eligible for New South Wales State Superannuation.

The appointee will also be given the title of Professor or Associate Professor by the University of Sydney and will have access to research facilities in the Department of Pathology within the University.

Fares and Removal Expenses: Assistance towards fares, removal and initial accommodation expenses for interstate or overseas applicants will be considered.

Enquiries: Dr T. Rennie, Health Commission of New South Wales, 9-13 Young Street, Sydney, NSW 2000, re appointment as Director, Division of Forensic Medicine, under New South Wales Public Service Act; and to the Dean, Faculty of Medicine, University of Sydney, NSW 2006, re appointment as Professor or Associate Professor in the Department of Pathology, University of Sydney.

Applications giving full details of qualifications and experience and names of three referees by **October 3, 1978** to the Registrar, University of Sydney, NSW 2006, Australia. Information also from Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. 7098(A)

THE UNIVERSITY OF MANCHESTER INSTITUTE OF SCIENCE AND TECHNOLOGY ASSISTANT DIRECTOR OF THE POLLUTION RESEARCH UNIT

Applications are invited from suitably qualified engineers or scientists to lead the U.M.I.S.T. group of the Pollution Research Unit organised jointly with the University of Manchester. The successful candidate should ideally be able to undertake the following:—

- (1) Fundamental or applied research in the pollution field.
- (2) Contributions to the organisation and teaching of an existing successful M.Sc. course.
- (3) Short courses.
- (4) Consultancy and test work for industry.

Industrial experience would be an advantage. Excellence in all the above fields is not essential provided the applicant possesses the paramount ability to motivate and coordinate interdisciplinary activities within U.M.I.S.T. and the University, while cooperating fully with the Director, who is located at the University.

Salary will be within scale £7,074 p.a. to £8,730 p.a. (under review).

Applications and further particulars may be obtained from the Registrar, U.M.I.S.T., P.O. Box 88, Manchester M60 1QD. Quote Reference PR/153/A1. Closing date: October 13, 1978. 7110(A)

LANCASHIRE COLLEGE OF AGRICULTURE SCHOOLS LIAISON OFFICER

Applications are invited for the newly created post of Schools Liaison Officer at the Lancashire College of Agriculture. Salary will be on the Lecturer Grade 2 scale for Farm Institute staff up to a maximum of £7,251 p.a. dependent upon qualifications and experience. Car allowance payable. The person appointed will be concerned with the establishment and development of a school liaison unit and knowledge or experience of relevant school work would be an advantage. Further details and forms of application are available from:—

The Principal Agricultural Officer,
Lancashire College of Agriculture,
Myerscough Hall,
Bilsborrow,
Preston PR3 0RY,
Lancashire.

Telephone: BROCK (0995) 4061.

to whom completed applications should be returned not later than October 9, 1978. 7112(A)

CHANGE OF ADDRESS

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TELEPHONE: 01 831-6901

UNIVERSITY OF RHODESIA
FACULTY OF ENGINEERING
CHAIR OF MECHANICAL
ENGINEERING
LECTURESHIPS
IN ELECTRICAL
AND MECHANICAL
ENGINEERING

Applications are invited from persons with engineering degrees and post-graduation experience for:—

Professor and Head of Department of Mechanical Engineering.

Lecturer or Senior Lecturer in the Department of Electric and Power Engineering (preferably with experience in Electric Power Systems, Electrical Machines, and Electromagnetics), and in the **Department of Mechanical Engineering** (preferably with experience in Thermodynamics, Heat Transfer and Mechanical Design).

Salary Scales (Approx. Stg. equiv.):

Professor
£10,461 by 394 to £12,431

Senior Lecturer
£7,606 by 295 to £8,491 by 313 to £10,056

Lecturer Grade I
£6,899 by 259 to £7,935

Lecturer Grade II
£4,260 by 224 to £5,380 by 259 to £6,675

Subject to University permission limited extra earnings for consultancy may be retained in full. Above this limitation they are shared with the University.

Commencing salary according to qualification and experience. Family passages and allowance for transport if effects; installation loan; travel allowance for Sabbatical and Biennial Professor) or Triennial (Lecturers) visits; superannuation and medical aid schemes. For persons recruited outside Rhodesia, unfurnished accommodation in or near the Campus, and within easy reach of good Junior and Senior schools, is guaranteed for up to three years.

Appointments: These may be on permanent pensionable terms or for a fixed term of 1 or 2 years in the first instance. In the case of 1 and 2 year contracts a non-pensionable salary is payable as above plus a 10% supplement. Unless born in Rhodesia ex-atriates on contract are exempted from Military Service.

Applications: One copy giving personal particulars (including full names, place and date of birth), qualifications, fields of special interest, experience and publications, and giving names and addresses of three referees should be sent by **November 17, 1978** to The Senior Assistant Registrar (Engineering), University of Rhodesia, P.O. Box 417, Mount Pleasant, Salisbury, Rhodesia, from whom further particulars may be obtained. Overseas applicants please send an additional copy to: the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, from whom further particulars may also be obtained. Applicants should state in their applications the earliest date available and also the preferred date.

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 233-4143) or their nearest British Consular Office.

7072(A)

A CAREER IN APPLIED PHYSICS?

Opportunity for a Scientific Officer at the Daresbury Laboratory.

Daresbury Laboratory, an establishment of the Science Research Council situated in the north Cheshire countryside, is constructing the world's first high energy (2 GeV) electron storage ring specifically designed to provide a large number of synchrotron radiation beams. This new National Facility is to be commissioned in 1980 and will be used by well over 100 British and foreign experimental teams from many scientific disciplines including physics, chemistry, biology and materials science.

The vacancy

The Synchrotron Radiation Source (SRS) Group has a vacancy for a young physicist to join the team responsible for the construction, operation and development of the SRS and for the powerful computer network which controls the machine. The Group's activities cover a wide area of applied physics often involving development work at the frontiers of technology. Further information about the post can be obtained from Mike Poole (Ext. 256) on the number shown below.

Who do we need?

We are looking for a recent graduate, of either sex, with a good honours degree in physics who could contribute to the SRS Group's challenging work programme, both as an active member of a team and as an individual using his/her own initiative. For example the person appointed would be expected to contribute to theoretical and experimental studies of the limitations imposed on beam intensity by the behaviour of electron bunches in a metal vacuum chamber.

Interested?

Applications are sought from graduates who wish to embark on an interesting and rewarding career in a field of research and development whose importance is growing rapidly. An appointment will be made at Scientific Officer level, according to qualifications and experience on a scale £2,839 to £4,415 per annum. An applicant with an upper second class honours degree and no experience might expect a starting salary of £3,294 per annum. There is a non-contributory superannuation scheme, a generous leave allowance and a flexible working hours scheme.

Closing date: 1 October 1978

To request an application form, telephone 0925-65000, Ext. 467, quoting reference number DL/638/ , or write to:

David Mitcham
Personnel Officer

DARESBUURY LABORATORY
Science Research Council
Daresbury, Warrington WA4 4AD

7074(A)

CHANGE OF ADDRESS

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TELEPHONE : 01 831-6901

Chemical Process Development

Worthing

Two organic chemists are required to work on the development and scale-up of processes for the production of existing and prospective products at our £multi-million manufacturing facility at Worthing.

Applicants, male or female, should possess either a PhD or a good honours degree together with an awareness of current technologies. Some relevant industrial experience would be advantageous. Good communication skills are called for as close liaison will be necessary with Production, Quality Assurance, Engineering and Research departments.

We are offering competitive salaries and excellent fringe benefits including free pension and life assurance, plus outstanding sports and social facilities. Working conditions at Worthing are attractive and the laboratories are extremely well equipped with the most up to date analytical and process instrumentation.

Please telephone or write for an application form to **Mr. A. D. Caws, Senior Personnel Officer, Beecham Pharmaceuticals, Clarendon Road, Worthing, West Sussex, BN14 8QH. Telephone: Worthing 39900 Extension 233.**

7113(A)

Beecham Pharmaceuticals

MEDICAL RESEARCH COUNCIL CYCLOTRON UNIT

requires a

PHYSICIST or an ENGINEER

to join a team investigating the potential of hyperthermia in the treatment of cancer. The successful candidate will be responsible for the development of methods of inducing local hyperthermia both in experimental animals and in man. At least 3 years postdoctoral experience in microwave or RF heating technology will be required. Active collaboration with biological scientists and physicians is an essential part of the programme, therefore the successful candidate will have a genuine interest in biology and medicine.

The post is a five-year limited term appointment with salary in the range of £4,815 to £6,404 (inclusive of London Weighting) depending upon age and experience.

Applications, with full curriculum vitae to:

Miss A. Pires
M.R.C. Cyclotron Unit
Hammersmith Hospital
Ducane Road, LONDON W12 0HS
Tel: 01-743 4594, Ext. 103

7131(A)

ULSTER POLYTECHNIC

Faculty of Science

RESEARCH OFFICER

SCHOOL OF PHYSICAL SCIENCE

Salary Scale : £3,732 to £4,632

A Research Officer is required to assist a group working on the synthesis and bioassay of spirolactone fungicides. Applicants must have a good honours degree or equivalent in Chemistry and should have relevant research or industrial experience. Postgraduate qualifications would be an added advantage.

The Polytechnic is a direct grant institution with an independent Board of Governors. It opened in 1971 and now has a student population of some 7,100. It has extensive new purpose-built accommodation, including 750 residential places on the 114 acre campus overlooking the sea at Jordanstown, a pleasant and quiet residential area. There is a scheme of assistance with removal.

Further particulars and application forms which must be returned by October 16 may be obtained by telephoning Whiteabbey (0231) 65131 ext. 2243 or by writing to:

The Establishment Officer, Ulster Polytechnic, Shore Road, Newtownabbey, Co. Antrim BT37 0QB. 7077(A)



INVERESK RESEARCH INTERNATIONAL

RESEARCH SCIENTIST

Applications are invited for the post of Research Scientist within the section of *in vitro* Toxicology. The successful candidate will be expected to carry out established tests for transformation, mutation and DNA repair in mammalian cell cultures, to participate in research and development of new tissue culture techniques for the detection of mutagens, carcinogens and teratogens. Candidates should have a biological or biochemical background, with postgraduate experience in tissue culture, and have the ability ultimately to lead projects and supervise the daily routine within tissue culture.

I.R.I. is located near Edinburgh, within easy reach of the city and surrounding area. Relocation assistance will be provided if required and there is a full range of staff benefits. Application forms, Ref. 2314, are available from Miss J. M. Dunbar, Inveresk Research International, Inveresk Gate, Musselburgh EH21 7UB. 7111(A)

EUROPEAN MOLECULAR BIOLOGY ORGANISATION CHROMATIN WORKSHOP

23-26 March 1979

Courmayeur, Italy

This Workshop is intended as a forum for investigators working in this field (including graduate students). Numbers will be restricted. There will be a limited number of grants to defray costs of registration and accommodation. The main theme of the Workshop will be concerned with transcription and its control.

Applicants should send a brief curriculum vitae, a statement of interest in the field and, if desired, letters of support, before October 31, 1978, to:

Dr J. Paul,
The Beatson Institute for
Cancer Research,
Garscube Estate,
Switchback Road,
Bearsden, GLASGOW G61 1BD,
Scotland. 7138(A)

ST. MARY'S HOSPITAL MEDICAL SCHOOL (University of London)

Paddington, London W2 1PG
DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT GRADE 1A

required to work with Dr P. Millburn and Dr P. C. Hirom on the entero-hepatic circulation of carcinogens and anti-cancer agents.

Appointment for up to 3 years starting October 1978/November 1978. Salary within scale £3,685 to £4,611 p.a. plus £450 p.a. London Allowance, depending on age and experience.

Informal enquiries to Dr Millburn (Tel: (01)723 1252 Ext. 118). Curriculum vitae with names and addresses of at least two referees to The Secretary at above address quoting Ref RA/BIO/N. 7127(A)

CHANGE OF ADDRESS

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TELEPHONE : 01 831-6901

Cardiac Research

A Senior appointment with ICI

Cardiac research which is one of the most important aspects of research at ICI has resulted in an extensive range of successful drugs, in particular, beta-blockers and lipid lowering agents.

We are now actively extending our cardiac research facilities to cover survival after heart attack—the survival of the myocardial cell under ischaemic conditions.

Consequently we are looking for a person to head up this project which has the full support of the Company's extensive resources. It is envisaged that the right candidate will have considerable experience in cardiac research.

You will be free to determine the approaches to be used and will lead a multi-disciplinary team of experienced researchers. You should be qualified in medicine, physiology or

pharmacology and have the ambition to further your career in research.

Remuneration and other benefits, including relocation expenses, are designed to attract candidates of high calibre. The post is based at our modern research establishment located in beautiful parkland in Cheshire. Residential, recreational and school facilities in the area are excellent.

Please reply, enclosing your curriculum vitae to:—



J W Eardley,
Personnel Department,
ICI (Pharmaceuticals Division) Limited,
Mereside,
Alderley Park,
Macclesfield,
Cheshire SK10 4TG.

7062(A)

DIRECTOR CANCER RESEARCH GROUP

The University of Western Ontario and the National Cancer Institute of Canada invite applications for the post of Director of a cancer research group. The present laboratories, which are fully equipped to undertake research on most problems in modern biology, occupy approximately 10,000 sq. ft. and are joined to the Health Sciences Centre and the University Hospital. Support for the research program and the salaries of senior staff members is provided by the National Cancer Institute of Canada. It is customary for senior staff members (usually five) to hold academic appointments, to be eligible to accept graduate students and to accept a modest teaching load. The salary will be dependent on experience and on levels prevailing at the University of Western Ontario. The deadline for receipt of applications is January 1, 1979 and the appointee could commence duties as early as July 1, 1979. Applications and enquiries should be addressed to:

Dr O. H. Warwick
Chairman, Selection Committee
c/o Office of Vice-President,
Health Sciences
University of Western Ontario
London, Ontario,
Canada N6A 5C1. 7084(A)

RESEARCH ASSISTANT or ASSOCIATE in charge of COMPUTING FACILITIES at a Medical Research Council Unit, Cambridge

The appointee will be required to manage and develop a computer system (PDP 8, 11 and 12) used for storing and analysing behavioural data, and to design and build special research equipment.

Applicants with relevant electrical engineering or electronics qualifications and some experience in one or more of these fields will be preferred.

- (i) interfacing mini computers preferably PDP's;
- (ii) using operating systems (preferably RT-11);
- (iii) computer maintenance.

Some programming ability (especially in assembler code) also an asset.

The appointment is on a Research Assistant (£2,956 to £5,058) or Research Associate (£4,365 to £5,730) scale, depending on age and experience.

Applicants should write to The Director, MRC Unit on the Development and Integration of Behaviour, University Sub-Department of Animal Behaviour, Madingley, Cambridge CB3 8AA. 7108(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF ZOOLOGY TECHNICIAN (ELECTRON MICROSCOPIST)

To work in the Department Electron Microscope Unit. Candidates should have experience or interest in Electron Microscopy and must possess O.N.C., H.N.C. or degree qualifications. Salary according to qualifications and experience in a range up to £3,720 per annum.

Application forms may be obtained from The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref RV/925/N 7109(A)

UNIVERSITY OF SOUTHAMPTON

Applications are invited for NRDC funded Postdoctoral Research Assistantships in Organic Electrochemistry to develop commercially viable electrochemical syntheses using novel concepts for the trapping of reaction intermediates. This project will cover both fundamental and applied aspects. Applicants should have research experience in either organic chemistry or electrochemistry.

Salaries will be in the range £3,883 to £4,882 per annum plus U.S.S. benefits.

Applications, giving a curriculum vitae and the names of two referees, should be sent to Dr J. M. Mellor and Dr A. Bewick, Department of Chemistry, The University, Southampton SO9 5NH. Please quote Ref MA1/2/Na. 7114(A)

SCIENTIFIC OFFICER/ HIGHER SCIENTIFIC OFFICER PHYSIOLOGY DEPARTMENT

Applications are invited from recent graduates or those holding suitable qualifications for the above post. The person appointed will be required to head a small team studying the significance of various morphological and physiological attributes for the water economy of cereal crops. The emphasis in the research will be on experimentation in field plots sheltered from rain by mobile plot covers. The work will require the determination of water use by the experimental crops and studies of their stomatal behaviour and radiation balance. Applicants should have an interest and preferably experience in the physical aspects of water movement through the soil and plant to the atmosphere. They should have a 1st or Upper 2nd Class Honours Degree or equivalent in a biological subject.

The appointment will be as Higher Scientific Officer (£4,101 p.a. to £5,448 p.a.) or Scientific Officer (£2,839 p.a. to £4,415 p.a.) according to qualifications and experience. At least two years postgraduate research experience is required for appointment as Higher Scientific Officer. Non-contributory pension scheme.

Further particulars may be obtained from the Establishments Officer, Plant Breeding Institute, Maris Lane, Trumpington, Cambridge CB2 2LQ and applications, with curriculum vitae and the names of three referees, should be sent to him by September 29 1978. 7085(A)

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- to assist management in determining the R & D effort required for successful registrations;
- to agree the resulting planning schedules with the management;
- to follow up work and insure that forecasts are met;
- to insure the availability of the required registration material at the dates forecast in the planning schedules;
- to assume responsibility for the format and presentation of the registration dossiers as required for various governments' regulatory agencies.

He/she will have:

- previous experience in this field, and/or
- a post graduate qualification (eg M.P.S. or Ph.D.)

High salary will be offered to the successful candidate. Please reply to Box No. 7118(A), 3 Dyers Building, Holborn EC1. 7118(A)

Scottish Institute of Agricultural Engineering
SOILS SECTION

SENIOR SCIENTIFIC OFFICER/PRINCIPAL
SCIENTIFIC OFFICER (Ref. AG/18/A)

(Re-Advertisement)

Applications are invited for the post of Senior Scientific Officer/Principal Scientific Officer to lead a team investigating the effects of agricultural machinery on the physical properties of field soils with particular reference to cultivations, zero-tillage and compaction.

QUALIFICATIONS: A first or upper second-class Honours Degree, and preferably a higher Degree, or equivalent qualifications in a relevant branch of Science or Engineering. The applicant should have had considerable experience of research in soil physics or soil mechanics.

SALARY SCALES: Appointment in the grade of Senior Scientific Officer (£5,154 to £6,898) or Principal Scientific Officer (£6,609 to £8,461) according to qualifications and experience. Non-contributory superannuation.

Application forms and further information from:—

The Secretary, Scottish Institute of Agricultural Engineering,
Bush Estate, Penicuik, Midlothian EH26 0PH.

to whom applications should be submitted by October 6, 1978. 7120(A)

THE HANNAH RESEARCH INSTITUTE HIGHER/SENIOR SCIENTIFIC OFFICER

A vacancy exists for a Higher/Senior Scientific Officer in the Physiology Department, to join a team engaged in studying the mechanism and control of milk secretion.

Applicants should possess a First or Upper Second Class Honours Degree in a relevant discipline with, preferably, a Ph.D.

Salary will be in the range of £5,101 to £5,448 for H.S.O. and £5,154 to £6,898 for S.S.O. At least two years relevant postgraduate experience is required for appointment to H.S.O. and at least four years appointment to S.S.O. Non-contributory superannuation scheme in operation.

Further particulars may be obtained from: The Secretary, The Hannah Research Institute, Ayr KA6 5HL, to whom applications, which should include a curriculum vitae, list of publications, and the names and addresses of three referees, should be sent by October 6, 1978. 7139(A)

UNIVERSITY OF GLASGOW DEPARTMENTS OF SURGERY AND BIOCHEMISTRY Glasgow Royal Infirmary RESEARCH ASSISTANT

Applications are invited for the post of RESEARCH ASSISTANT in an interdisciplinary group investigating the production of foetal antigens by human tumours. The post is suitable for a graduate in biochemistry or related subject. Experience in radio-immunoassay techniques would be an advantage. The successful candidate will work in the new Department of Biochemistry, Glasgow Royal Infirmary. The appointment is funded by the Cancer Research Campaign and is tenable for one year. The salary will be within the University Research Assistant scale (£3,660 to £4,130 per annum presently under revision). The appropriate superannuation scheme applies. Starting date by agreement.

Further information can be obtained from Dr J. G. Ratcliffe, Department of Biochemistry, Royal Infirmary, Glasgow G4 0SF (Tel. 041 552 3535, extension 5275) to whom applications including curriculum vitae and the names of two referees should be sent within 2 weeks of the appearance of this advertisement.

In reply please quote Ref. No. 4241(M). 7078(A)

BEDFORD COLLEGE (University of London) DEPARTMENT OF GEOGRAPHY RESEARCH ASSISTANT Limestone Weathering in Urban Environments

Applications are invited for RESEARCH ASSISTANTSHIP to be funded by the Building Research Establishment (Garston) to initiate mainly through literature and project reviews, a study of the mechanisms of limestone weathering in urban environments. Applicants should preferably be qualified in one or more of the following fields: chemistry, geochemistry, environmental science, or physical geography; ability to read German and French an advantage. European travel possible. The appointment is initially for 6 months, and may be extended for a further 6 months.

Gross salary: £3,834 p.a.
Applications to Professor R. L. Cooke, Bedford College, Regent's Park, London NW1 4NS, by October 3, 1978. 7137(A)

FRESHWATER BIOLOGICAL ASSOCIATION WINDERMERE LABORATORY ASSISTANT LIBRARIAN/ INFORMATION SCIENTIST

Applications are invited from graduates in information science, or science graduates with a qualification in Librarianship, for the above post which is to strengthen the Association's library and information services. The post will be graded as Assistant Librarian (£3,113 to £3,579 per annum) and conditions of service will be generally similar to those of the Civil Service. Full details and application form may be obtained from the Secretary, Freshwater Biological Association, The Ferry House, Ambleside Cumbria LA22 0LP. Closing date September 30, 1978. 7025(A)

UNIVERSITY OF MINNESOTA DEPARTMENT OF ASTRONOMY Applications are invited for a POSTDOCTORAL RESEARCH ASSOCIATE POSITION

in the

Department of Astronomy

Candidates should be experienced in radio interferometry and prepared to conduct and collaborate in research using the VLA radio telescope. The appointee will be expected to establish an initial VLA map analysis system and to participate in development plan for a permanent facility at the University of Minnesota. The position is available for one year, beginning on January 2, 1979. The possibility of an extension of the appointment for a second year is under consideration.

Candidates should send a letter of application, including a curriculum vitae and bibliography, and the name of three references who have been asked to submit letters of recommendation to Dr W. B. Burton, Chairman Department of Astronomy, University of Minnesota, 116 Church St. S.E. Minneapolis, Minnesota 55455. The deadline for receipt of applications is October 31, 1978. The University of Minnesota is an equal opportunity educator and employer. 7096(A)

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ST GEORGE'S HOSPITAL MEDICAL SCHOOL (University of London) POSTDOCTORAL RESEARCH ASSISTANT (Biochemistry)

required for the last 18 months of a M.R.C. project under Dr M. J. Clemens (01-672 1255, Extension 4750) on the nutritional regulation of protein synthesis in cultured animal cells and cell-free systems. Salary up to £6,317 p.a. plus £450 p.a. London allowance.

Applications with names of two referees to the Establishment Officer, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE. Closing date October 9. 7117(A)

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NATIONAL COUNCIL FOR SCIENTIFIC RESEARCH

Applications are invited for the following vacant posts in Building Research Unit:

1. Soil Mechanics and Foundation Engineer

Qualification: Degree in civil engineering and relevant experience.

Duties: To undertake soil surveys and site investigations with percussion and rotary drilling rigs and laboratory testing of soils for foundation works.

2. Materials Engineer/ Scientist

Qualifications: Degree in Materials Engineering Science with a minimum of five years experience in research and development work.

Duties: Successful candidate will be required to initiate and carry out research and development work on the utilisation of local raw materials in the production of soft boards or development of alternative roofing materials for low cost housing, and to provide technical advice on the production and usage of building materials.

The above-mentioned post are required to be filled immediately and not later than April 1979.

Salary will be according to qualifications and experience on the following scales:

Senior Principal Professional Officer: K7,812 by 204 to K8,016 by 216 to K8,232.

Principal Professional Officer: K6,756 by 216 to K6,972 by 288 to K7,200 by 204 to K7,608.

Senior Professional Officer: K5,424 by 288 to K5,712 by 300 to K6,012 by 312 to K6,324.

Applications giving full personal details, qualifications and experience and names and addresses of three referees should be submitted to:

The Secretary General,
National Council
for Scientific Research,
P.O. Box CH 158,
Lusaka,
Zambia.

7095(A)

UNIVERSITY COLLEGE OF NORTH WALES

Bangor

Applications are invited for the post of
LECTURER

IN MARINE CHEMISTRY

Applicants should have a good Honours Degree and experience and/or interests in inorganic solution of chemistry. Previous experience in the chemistry of sea water and/or natural waters would be an advantage.

The successful applicant will be expected to contribute to teaching undergraduate and postgraduate related courses, and to undertake research in the field of marine chemistry.

The appointment, commencing on October 1, 1978, or as soon as possible thereafter, will be to the lower portion of the scale £3,883 to £7,754 per annum.

Applications (two copies), giving details of qualifications and experience, together with the names and addresses of three referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, from whom further particulars may be obtained.

Closing date for applications: October 2, 1978. 7133(A)

AN FORAS TALUNTAIS

requires a

Agricultural Science Graduate

at it's Moore Park Research Centre, Fermoy, Co. Cork, to undertake research on the development of intensive dairy farming systems with particular emphasis on grass-land production and utilisation.

ESSENTIAL: An Honours University Degree or equivalent in Agricultural Science.

DESIRABLE: Suitable research experience.

Appointment up to and including Principal Research Officer level depending on qualifications and experience. Marriage and children's allowances, Non-contributory Pension Scheme and provision for widow's and orphan's pensions.

Application forms and further particulars available from the Personnel Department, (quote ref: Mor), An Foras Taluntais Headquarters, 19 Sandymount Avenue, Dublin 4. (Phone: 688188). Latest date for receipt of completed forms, Monday October 13, 1978. 7079(A)

AGRICULTURAL RESEARCH COUNCIL

Plant Physiologist

Applications are invited from Plant Physiologists who would like to join a small team of senior scientists who advise the Secretary of the Council on the scientific aspects of research administration.

The post is graded Senior Principal Scientific Officer with a salary scale of £10,043-£11,300 plus £465 for working in Central London. Superannuation is under a non-contributory scheme analogous to that of the Civil Service.

Applications from candidates with a good honours degree or equivalent qualification and a successful record of research should be by letter accompanied by a full curriculum vitae and the names of two referees.

Further particulars from Miss M. J. Gard,
Agricultural Research Council,
160 Great Portland Street,
London WIN 6DT.
Closing date: 20 October 1978.

7103(A)



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M.R.C. CLINICAL RESEARCH CENTRE

(Northwick Park Hospital)

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POST DOCTORAL CELL BIOLOGIST

required to work with Dr R. T. Dean on membrane function in cystic fibrosis cells with particular reference to enzyme secretion and intracellular lysosomal events. The work will be carried out under a grant awarded for three years by the Cystic Fibrosis Research Trust. The appointment will therefore be for three years on a salary scale starting from £4,155 plus £450 a year London allowance.

Application form and further details obtainable from Mrs J. Tucker-Bull, 01-864 5311, extension 2685.

Closing date October 7, 1978. Please quote Ref. 104/1/GH. 7107(A)

FELLOWSHIPS

SENIOR VISITING FELLOWSHIP PROGRAM

Centre for
Interdisciplinary Studies
in Chemical Physics
The University
of Western Ontario
London, Ontario,
Canada N6A 3K7

The Centre for Chemical Physics at The University of Western Ontario invites applications for its Senior Visiting Fellowship Program from established scientists working in areas that bridge two or more of the disciplines of applied mathematics, physics, chemistry, engineering, botany, biophysics, nuclear medicine and radiation oncology. The Fellowships are tenable for a period of up to 12 months, and provide funds for travel, partial or complete living support at U.W.O., and some research support. During the tenure of their Fellowships, visitors will be expected to work in conjunction with Centre members.

Interested persons are asked to write to:



8/8/78 The Chairman, Visiting
Fellows Committee,
Centre for
Interdisciplinary
Studies in Chemical
Physics,
The University of
Western Ontario,
London, Ontario,
Canada N6A 3K7

for application forms and additional
information. Applications for 1979
must be received no later than
December 15, 1978. 7081(E)

TWO POSTDOCTORAL FELLOWSHIPS IN THE EARTH AND PLANETARY SCIENCES

The Lamont-Doherty Geological Observatory of Columbia University invites scientists interested in any field of the earth and planetary sciences to apply for the following Fellowships.

Two Postdoctoral Fellowships, each awarded for a period of one year (extendable to two years in special instances) beginning in September, 1979, with a stipend of \$16,500 per annum. Completed applications are to be returned by January 15, 1979. Application forms may be obtained by writing to the Director, Lamont-Doherty Geological Observatory, Palisades, New York, USA 10964.

Award announcements will be made February 28, 1979. 6099(E)



Southampton THE UNIVERSITY

THE HARTLEY FELLOWSHIPS

Applications are invited for three Fellowships normally tenable from October 1, 1979, for up to two years research, in any field for which facilities are available within the Faculties of Arts, Science, Engineering and Applied Science, Social Sciences, Law, Education, Medicine and Mathematical Studies.

Fellows will normally be appointed from persons of high promise whose records show evidence of capacity for original research, and who have up to five years postdoctoral experience or equivalent research record.

Salary will be related to age and experience and be within nationally agreed ranges for research staff in universities. (No provision for travel or removal expenses).

The closing date for applications for the 1979 competition is November 1, 1978.

Details and application forms from Mr E. D. Gordon, The University, Southampton SO9 5NH. Please quote ref. MA/1/Na. 7076(E)

UNIVERSITY COLLEGE OF WALES Welsh Plant Breeding Station

Applications are invited for a

RESEARCH FELLOWSHIP

commencing November 1, 1978 to work on the use of multiline varieties of barley for the control of barley mildew.

The fellowship which is funded by the Home Grown Cereals Authority, will be given for three years, and will carry an allowance of £3,254 to £3,527 per annum.

Applicants should have postgraduate experience in plant pathology, preferably in epidemiology.

Applications together with curriculum vitae and the names of three referees should be sent to the Secretary, Welsh Plant Breeding Station, Plas Gogerddan, Aberystwyth, Dyfed, by Monday, October 2, 1978, from whom further details are available on request. 7086(E)

IMPERIAL CANCER RESEARCH FUND RESEARCH FELLOWSHIP

A Postdoctoral Fellow required as soon as possible for an appointment of two years with possible extension to three years. The work will be on the mechanisms by which host cells control the expression of tumour-inducing viruses. Preference will be given to candidates familiar with nucleic acid hybridisation and allied techniques.

Salary with entry according to qualifications and experience within range £4,815 to £6,180 incl.

Applications with curriculum vitae and names of two referees, should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX by October 27, 1978, quoting reference 263/78. 7059(E)

UNIVERSITY OF SOUTHAMPTON DEPARTMENT OF OCEANOGRAPHY

Applications are invited for a POSTDOCTORAL FELLOWSHIP

to work on plankton photosynthesis in an estuary. The successful applicant will be expected to undertake field and theoretic studies to develop a model for photosynthesis for the Medway Estuary. The work will be undertaken in conjunction with the Water Research Centre. The fellowship will be tenable from October 1, 1978 to December 31, 1979.

Applications, giving the names of two referees, should be sent as soon as possible to Dr P. J. Williams, Department of Oceanography, The University, Highfield, Southampton SO9 5NH, from whom further particulars may be obtained.

Salary range: £3,883 to £6,080 per annum plus U.S.S. benefits.

Please quote ref. MA1/2/Na. 7088(E)

THE UNIVERSITY OF SHEFFIELD POSTGRADUATE SCHOOL OF LIBRARIANSHIP AND INFORMATION SCIENCE JUNIOR

RESEARCH FELLOWSHIP

Applications for the above post are invited from men and women graduates with good honours degrees interested in undertaking research in librarianship or information science. The Fellow will be required to undertake light teaching duties, and to undertake research (towards a Ph.D. degree). The School's research interests are wide.

Tenable annually for 3 years, initial salary in range £3,189 to £3,895 a year.

Particulars from the Registrar and Secretary, The University, Sheffield S10 2TN, to whom applications (5 copies) should be sent by October 9, 1978. Informal enquiries to Professor M. F. Lynch, P.G.S.L.I.S. Quote Ref. R197/G. 7121(E)

STUDENTSHIPS

UNIVERSITY OF WARWICK RESEARCH STUDENTSHIP IN BIOCHEMISTRY

Applications are invited for a three-year research studentship in the Department of Chemistry and Molecular Sciences, sponsored by the Science Research Council under the C.A.S.E. scheme. The project, with Dr B. E. P. Swoboda will involve study of the mechanisms of action of cytosine arabinoside, currently used in cancer chemotherapy including the effects of the drug on DNA synthesis. Candidates must possess either a first class or an upper second class degree in Biochemistry or a related subject. Applications (two copies) giving qualifications and experience and naming two referees should be sent to the Academic Registrar, University of Warwick, Coventry CV4 7AL, as soon as possible, quoting Ref. No. PG/5/71 7082(A)

SCHOLARSHIPS

THE ROYAL SOCIETY GOVERNMENT GRANT FOR SCIENTIFIC INVESTIGATIONS

Applications for grants from the third allotment of the Government Grant for Scientific Investigations for the year 1978 should be made not later than November 15, 1978 on forms of application to be obtained from the Executive Secretary of the Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.

Applicants must be British subject domiciled in the United Kingdom. Grants made be made to promote an support research in science and to assist scientific expeditions and collections; but not for personal maintenance, payment of stipends or to aid scientific publications. 7116(H)

THE ROYAL SOCIETY SCIENTIFIC PUBLICATION GRANT

Applications for grants from the second allotment of the Scientific Publications Grant for 1978 should be made not later than November 8, 1978 on forms obtainable from the Executive Secretary, The Royal Society, Carlton House Terrace, London SW1 5AG. The grant is for the assistance of scientific publications in the United Kingdom, particularly, but not exclusively, those of scientific societies and institutions.

Applications are receivable only from societies and institutions in the United Kingdom and individuals of British nationality. 7115(H)

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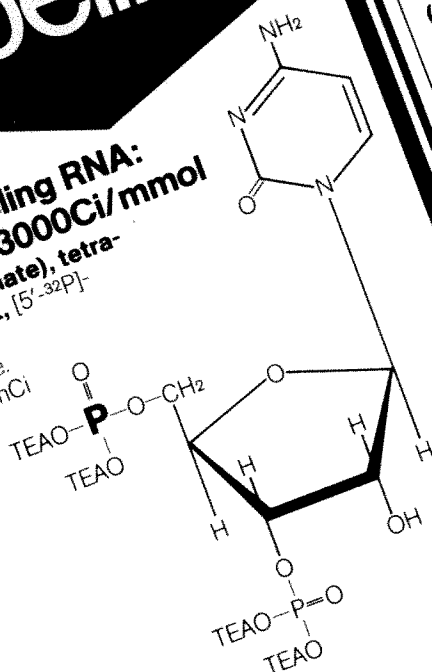
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 NEG-019 500μCi 1mCi



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 Also dCTP >300Ci/mmol
 dATP NEG-013X 500μCi 1mCi
 dGTP NEG-014X 500μCi 1mCi
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 Ethanol:water, 1:1, dry ice.

5' end labeling DNA and RNA:
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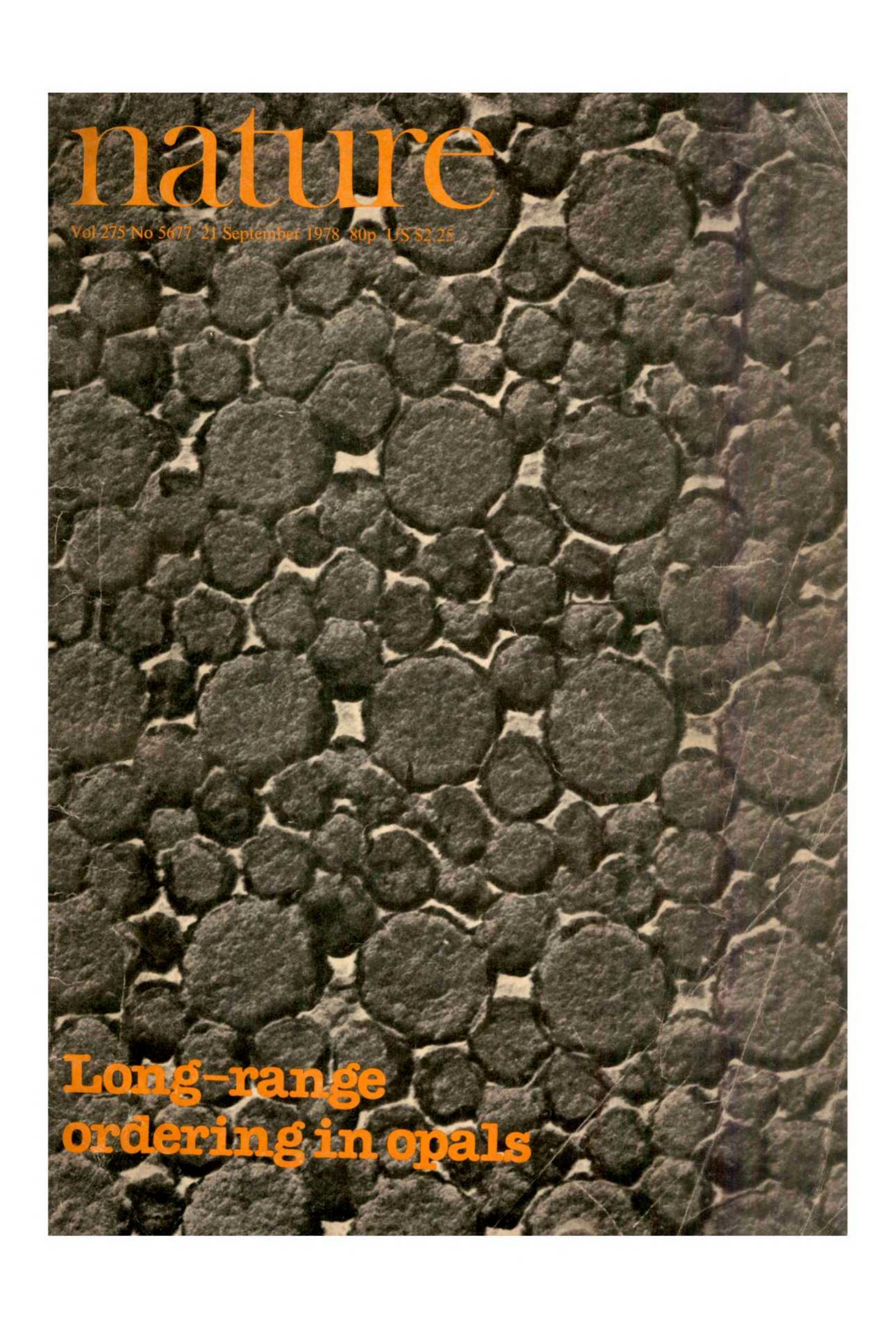
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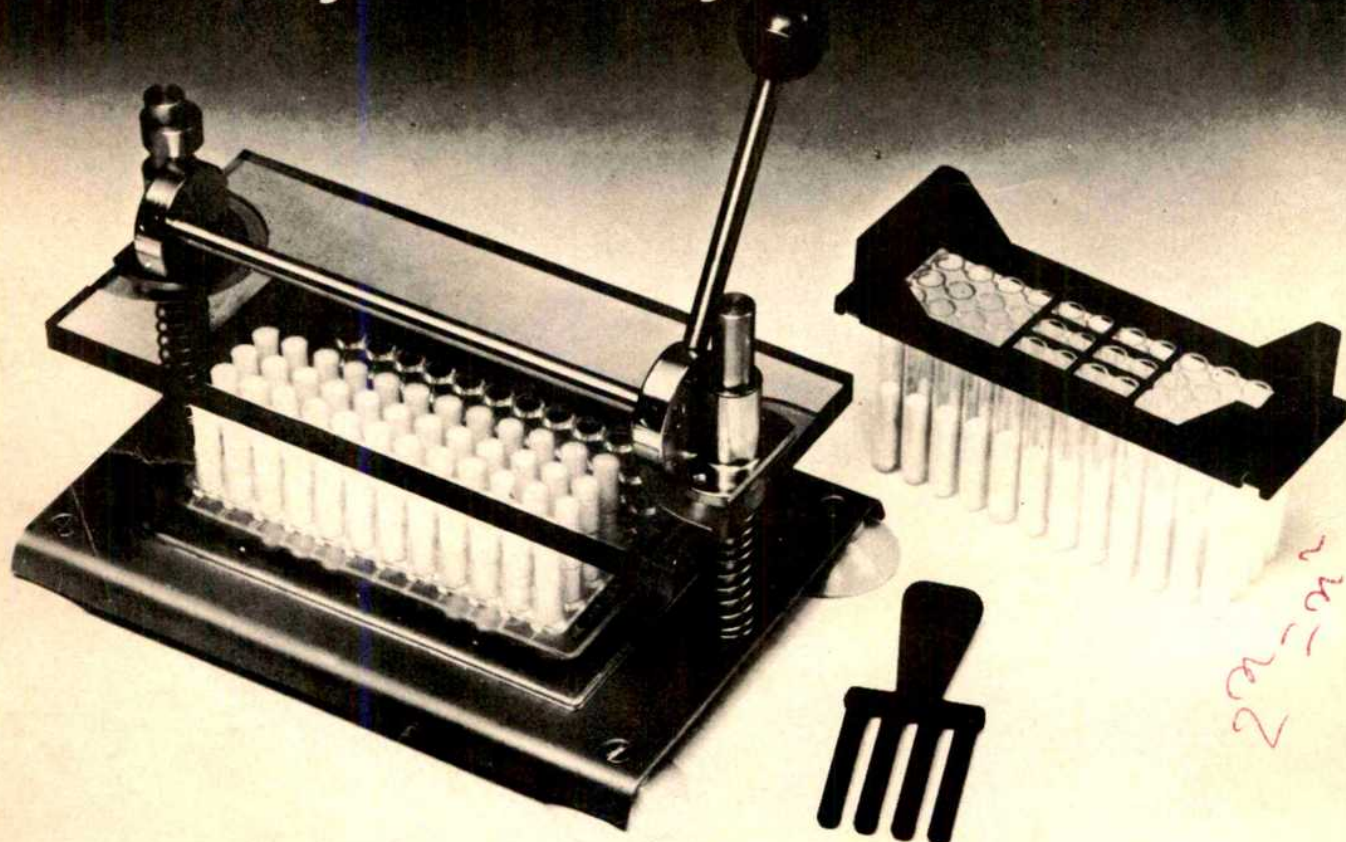
A high-magnification micrograph showing the characteristic 'peacock' pattern of opal. The image displays a dense, repeating array of dark, roughly circular or hexagonal domains, which are the silica spheres, separated by lighter-colored, irregular gaps. The overall texture is granular and highly ordered, illustrating the long-range periodic arrangement of the spheres.

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Cover picture

Ordered arrangements of two sizes of spheres of silica in a gem opal. The observation of long-range ordering in opal is reported on page 201.

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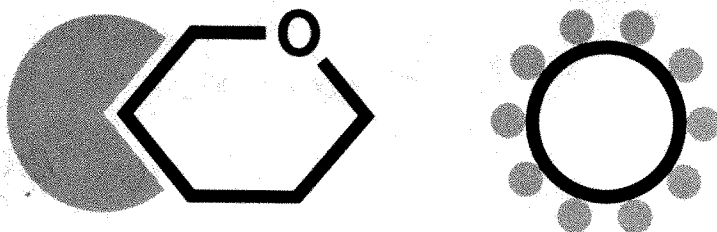
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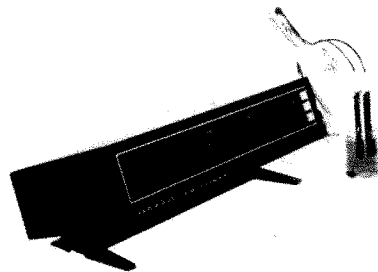
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ammonia in soils
ammonia in biological fluids
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calcium in serum
chloride in boiler feed water
chloride in foods
chloride in soils
chlorine
dissolved oxygen
copper in natural waters
cyanide in waste waters

fluoride in air and stack gases
fluoride in waste waters
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First things first in the Third World

AS THE national papers pile up in the headquarters of the United Nations Conference on Science and Technology for Development due now in less than a year's time, the pace of international meetings preparatory to the conference also grows. One of the most interesting, because of its wide ranging geographical representation, particularly of socialist countries, has been that organised by the World Federation of Scientific Workers in conjunction with the Union of Algerian Engineers and held in Algiers last week. The gathering, the responsibility of Professor J. M. Legay, showed rather clearly both the strengths and weaknesses of calling an international meeting to discuss a subject so large and so open to wide differences in interpretation.

Let us dispose of the weaknesses rapidly in order to concentrate on the strengths. The contribution to the conference by members of developed socialist countries was largely disappointing. Many from other parts of the world must have looked forward to discussion of socialist policies on development; they were given only vague theorising and totally uncritical self-advertisement in papers of almost uniform tedium. Example "The mission of structuring the overall problematique of social development into development complexes is to facilitate decisions in situations when concrete variants of sectoral development have to be derived from strategic goals or generally formulated concepts".

If there is one message that ought to get back to the governments of socialist countries—and indeed of many capitalist ones too—it is that the preparations for UNCSTD are making many scientists and technologists of the developing world much more critical of the statements of developed nations, and meaningless, self-satisfied or jargon-ridden papers will cut little ice.

That said, it is pleasing to be able to report that contributors from developing countries, and a few non-socialist countries, did have some significant and provocative things to present. It was clearly a hope of the organisers that the meeting would shed some light on scientists' responsibilities in the establishment of the New International Economic Order, that concept which also emerged from Algeria just five years ago and which is espoused with enthusiasm throughout much of the Third World as a way, *inter alia*, of obtaining control over natural resources and exerting more influence on the global monetary system. This new order is, however, not mentioned much in the capitalist world; as Professor E. H. S. Burhop, president of the Federation, put it, "the

overwhelming majority . . . in the industrialised capitalist countries . . . have never heard of the new order and are quite unprepared for the vast economic changes in their own countries it implies". The contributions that made the most impact, however, were not those that concentrated on praising the new order, or for that matter reviling the multinational corporations. Most effective were those papers that took a hard look at real problems in the developing world, admitted mistakes rather than tried to dole out blame, and made positive proposals for the alleviation of difficulties.

Two issues of importance seemed to emerge. The first was the very serious problem of effective use of intellectual resources in the developing world. Higher education in the Third World is easily seen as a status symbol, by governments and people alike. The more universities, the more PhDs, the closer the resemblance to western seats of learning, the better. But the further this path is trodden, the less it seems that trained scientists and technologists have an understanding of what people's needs are. This, of course, is not a phenomenon exclusive to the developing world, but whereas in the developed world we may have the luxury of debate as to how "relevant" university education need be, such a discussion in the developing world must surely be subservient to very visible national needs. And yet India, with more than a million technically qualified people, hardly seems to have harnessed this resource at all, and, in the words of a Pakistani participant, the role of science in the development of his country "has not been very significant".

There are many factors that have apparently contributed to a detachment of education from urgent national needs in many developing countries. The siren-call of developed-world science diverts too many into vain attempts at imitation. Poor experimental facilities drive many a good scientist into rather sterile theory. Service to the community through the provision of better water supplies or more efficient energy use is not seen to be very prestigious. There is a pressing need for those concerned with national science and educational policies to recognise that higher education and scientific research establishments can best serve development not by mimicry of the developed world but by a long cool consideration of local, often rural conditions where the potential for science-based improvements is still enormous.

The second issue of importance to come from the conference was that development which simply results in more

jobless cannot be acceptable. Higher productivity can well lead to unemployment, so it is most important that the aim, particularly within the rural sector, should not be for a mighty technological revolution which releases vast numbers of agricultural workers to flock to cities incapable of receiving them. The abundant manpower resources of many developing countries have to be handled with the greatest of care. One Bangladeshi contribution put this very succinctly: "in most cases our object is to upgrade simple rural technology to a slightly higher level of sophistication which improves the quality of life for the workers and creates more job opportunities." The emphasis, then, is on modest aims and on evolutionary development. In a notably thoughtful contribution, Dr H. Harada, Deputy Assistant Director-General for Science at Unesco, drew attention to the need to accept a slow and deliberate pace, in which a nation is at pains to develop science ahead of technology.

So what comes from such a conference? A final docu-

ment, necessarily general, of course. But maybe the germs of something more substantial. Numerous participants, while recognising that no New International Economic Order will come about without a correspondingly new national order, note nonetheless that there is enormous scope for international research centres to cope with developing world problems. Few of these problems respect national frontiers, nor are they such that questions of commercial secrecy should be involved. If there can be an International Centre for Theoretical Physics, specifically aimed to assist developing countries, is there not some sense in international, maybe regional centres on other scientific matters perhaps of a more immediate concern to development? A few exist already, of course, notably through the Canadian initiative of the International Development Research Centre, but there was little doubt in people's minds at Algiers that there was scope for more. As David Dickson reports on the following page this mood now seems widespread. □

Multinationals and development

Multinational corporations often come under criticism for their involvement in the developing world. But, argues Lewis Branscomb, Vice President and Chief Scientist of IBM, multinationals make major contributions to indigenous development.

THE United Nations Conference on Science and Technology for Development (UNCSTD) will look at the urgent need for indigenous capabilities in both developed and developing nations to mobilise science and technology in the cause of human problems. Poorer nations are understandably impatient with the slow and expensive process of institution building out of which emerged the education, research and industrial enterprises of the industrial democracies. But without the capacity to absorb, adapt and exploit technical knowledge, no amount of documented knowledge, patent licences or computer access will produce the economic results that are needed. How can this capacity be built?

National assistance agencies, international banks and private foundations can do a lot more than they have. Technical assistance programmes should be separated from security and capital assistance. Every prosperous nation has to find its own way to focus the energies of its universities and private sector establishments on institution building in cooperation with less developed countries. President Carter has made a farsighted proposal in his speech in Venezuela, proposing a government-sponsored foundation for technical cooperation to deal with this long-term need of the developing world.

Technical cooperation in education and research can look after many basic human needs, but industrial capability is only built up very slowly in this way. What is a more direct route? The fastest way to transfer technical capability from one nation to another is by exchange of the people with skills and experience, tools and procedures, and (when required) their financial resources and foreign market access. American industrial technology was built by direct foreign investment by European firms in Victorian times. Research was little known in 19th century American universities; the nation's industry was craft-oriented. Most of the population was in agriculture, which based its productivity more on the expanse of unexploited fertile land than on the cropping technology of Europe.

Why then are today's industrial enterprises which are

engaged in technologically-sophisticated operations in many countries (the "multinationals") so often regarded as part of the problem, when in fact they are a part of the solution?

When a responsible, modern multinational starts a business operation in a host developing country, it contributes to indigenous technical and managerial development in many ways. For example:

- By hiring and training host-country nationals for all levels of employment in the national subsidiary. Education and experience in industrial operations is gained in the community and is made successful under local conditions.
- Through technical cooperation of the multinational's local manufacturing plants with its plants and laboratories in other nations, a broad base of technical support is available in the event of technical difficulties.
- By obtaining materials, parts and assembly from local suppliers (IBM has 80,000 such vendors worldwide), a great deal of technological knowledge and experience is transferred, as the local company of the multinational works with each vendor to assure quality control of the product.
- As local skills develop and local markets justify expanded investment, more complex products can be undertaken by national subsidiaries and access to international markets expanded. In this way multinationals make important contributions to the balance of trade in many developing countries.
- Many products, such as communications equipment, trucks, machine tools and computers, not only contribute to national wealth directly but have large secondary benefits because their use in other sectors can increase productivity in the economy as a whole.

Sovereign nations must certainly look after the interests of their citizens by determining the rules regarding investments of skills and money within their borders. It is important that the leaders of those countries avail themselves of technical advice from those with industrial experience, to ensure that policies driven by economic objectives take into account technological realities. Transferring technology, even within the component parts of a single American or European enterprise, is not easy and requires time, training and extra expense. But above all it calls for a cooperative attitude by all the participants.

Let us hope that this is the outcome of the 1979 conference. □

The United Nations conference on Technical Cooperation between Developing Countries, which ended in Buenos Aires (right) last week, was a great success for third world countries, writes David Dickson

Camera Press



Developing countries to boost joint research

JOINT research projects between two or more developing countries are to receive greater emphasis, according to an ambitious 'plan of action' adopted last week by 138 member countries of the United Nations Development Programme. The plan was the main result of the conference on technical co-operation between developing countries (TCDC) which ended last week in Buenos Aires, Argentina.

The plan of action is based largely on suggestions made to the conference by the so-called 'Group of 77'—comprised now of 114 developing nations. The plan suggested that developing countries should "encourage existing national research and training centres to broaden their scope of activities to include programmes and projects which are of interest to several countries at a sub-regional, regional, and inter-regional level".

The conference also accepted a resolution submitted by Jamaica on behalf of the Group of 77 urging all developing countries to cooperate in strengthening existing research and training centres, with the intention of giving them "a multinational scope in the framework of TCDC" and where necessary establishing new institutions to achieve these ends.

And in order to prevent the plan of action being shelved as a well-meaning but ineffective document, the conference included in its recommendations specific directions for the way in which TCDC is to be encouraged and propagated, both within and outside the UN system.

Thus while rejecting as unnecessarily bureaucratic the idea of a new UN body with specific responsibilities for TCDC issues, the plan of action directs the UNDP to organise regular meetings—initially annual, subsequently biannual—or the planning ministers of member states to discuss progress towards TCDC goals.

Significantly the plan indicates that funds currently allocated by UNDP for regional use should now be devoted "to the maximum extent possible" to TCDC projects. Funds allocated to inter-regional or global projects could be used for TCDC projects proposed

by two or more countries from different regions.

The significance of these decisions is that, while using international funds, the developing countries will be able to exert greater control over the way that money for technical projects is spent rather than the major impetus for the use of such funds coming from the developed countries.

Many delegates to the conference felt that the agreement on a regular meeting of planning ministers and the shift in the balance of control over technical projects had resulted in greater success for the conference than had previously been expected.

"The consensus that the conference was able to achieve on the plan of action represents a major victory for the developing countries", said Mr Abdel Razzak Meguid, Minister of Planning for Egypt and chairman of the main committee of the conference secretariat, in protest at the weakness of proposals put forward in an earlier draft of the plan.

"We have succeeded in making it acceptable to the North that it is in their interests to strengthen the 'horizontal' links between developing nations. That bridge is now not only accepted as a hope, but as a necessity if the world community is to survive".

However, few speakers at the conference pretended that TCDC was a new strategy. Background papers prepared for the conference, for example, described the complex network of overland trade routes that had existed between different countries in Africa before the arrival of European traders and colonialists.

Furthermore there has in recent years been a steady growth in joint research projects between various developing countries. The University of Chile, for example, is now co-ordinating a Latin American scheme for co-operation in postgraduate teaching and research in the biological sciences, which has already set up studies in areas such as the spread of plant viruses and the biological effects of working at high altitudes (such as in the mines of Peru).

What many delegates felt was new,

however, was the emphasis that the conference was able to place on the concept of TCDC not merely as defined by a limited set of bilateral or multilateral agreements, but as a basic strategy for achieving the goals of what has come to be known as the 'new economic order' and the joint attempts of developing countries to counter the economic dominance of the developed world.

Thus initially the conference had been conceived in relatively narrow terms as dealing primarily with issues relating to technological co-operation, partly at the instigation of the more advanced of the developing nations, many of whom—such as the host country Argentina—are looking for new outlets for their goods and technical services.

However, during the evolution of the conference, the concept of TCDC has gradually become much broader, covering radical approaches to education and research, through to strengthening transportation, communication and information links among developing countries.

"Since agreeing in principle on the need for a 'new economic order' in 1974 the United Nations has not done a single thing to translate a programme of action into an actionable programme. But I think that this conference has laid the framework for such an actionable programme" Mr Meguid said after the plan had been accepted by the conference.

Other delegates were similarly confident that the plan, which will now be placed before the General Assembly of the United Nations, provides a firm basis for a substantial shift in control over the content of development programmes from the developed to the developing nations, and that the regular meeting of planning ministers will provide considerable support for this trend.

As far as scientific research is concerned, the conference did not consider in detail any particular programmes or list of priorities, and many of the participants from specialised UN agencies such as the World Health Organisation and the Food and Agri-

cultural Organisation had to remain primarily as observers—much to the frustration of some who claimed considerable practical experience in the issues under discussion.

However, the plan of action makes a number of suggestions about how research should be conceived within the TCDC framework. For example, it speaks of the need for developing countries to combine research efforts

and share results “both with one another and with other developing countries by means of agreements on scientific and technological co-operation, strengthening national design, national laboratories, research centres and scientific and other institutions”.

Putting flesh on these ideals will be one of the main objectives of next year's United Nations Conference on Science and Technology for Develop-

ment, which will take place in Vienna. But many delegates left Buenos Aires with the feeling that despite internal differences, the developing countries have found in the issue of technical co-operation a way in which they can work together in presenting concrete demands to the developed world, and that this in itself was a major step in bringing the new economic order closer to reality. □

US geneticists look to Europe for research facilities

RESEARCHERS at Genentech Inc, San Francisco, claimed at a Munich conference last week that they have induced bacteria to synthesise the two separate amino acid chains of human insulin—and to have combined these chains to make minute quantities of the hormone. Last year, the same team used a similar technique to induce bacteria to produce the hormone, somatostatin, and earlier this year, another team led by Walter Gilbert of Harvard University announced that they had persuaded bacteria to produce rat pro-insulin attached to the bacterial enzyme penicillinase.

Synthesis of the artificial insulin gene used by Genentech began at City of Hope Medical Centre, California late last year. Researchers there synthesised artificial genes consisting of the DNA sequence corresponding to the amino acid sequence of the A and B strands of human insulin. Six months later, they turned the synthetic genes over to David Goeddel and Dennis Kleid, biochemists at Genentech.

Goeddel and Kleid inserted the synthetic genes for the two strands of insulin into plasmids which were then introduced separately into *Escherichia coli*. The bacteria replicated and expressed the genes as the A and B amino acid chains. These were then mixed to produce a fraction of a milligram of insulin which, it is claimed, is similar to human insulin.

Biological tests for the activity of insulin, however, have yet to be done. Even though chemical tests and radio-immunoassay have indicated the presence of the molecule, some researchers question the claim that the insulin produced is the chemical equivalent of human insulin. This is because radio-immunoassay cannot distinguish between different insulin varieties. Also, the B-peptide chains produced during the recent work are impure, and some researchers feel that the amino acid analyses conducted so far may be insufficient to establish the presence of

the desired sequence.

The development of a new source of insulin for the treatment of diabetes has great practical importance. About 5% of diabetics are allergic to the animal insulin now used, and each patient requires 10 bovine pancreases per year at a cost of more than \$100. Bacterially produced insulin should eliminate allergic reactions and may be cheaper and easier to supply than animal insulin. Before the newly produced insulin could be marketed, however, it would have to undergo extensive animal and human tests which could take two to five years, though Genentech president Robert Swanson foresees no real difficulties in scaling up the procedure and passing the tests.

Such rapid success in developing a practical application of a technique raises a host of questions. The technique was discovered so recently that the original patent application has not yet been processed, and neither have revised safety regulations been implemented. Already the race for other commercial applications is hotly contested. New patents have been applied for, the breadth of the original patent remains unclear, and several leading American researchers are reportedly setting up shop in Europe to escape regulatory delays.

The original research leading to the procedure used in synthesising artificial genes was conducted with funds from the National Cancer Institute, the

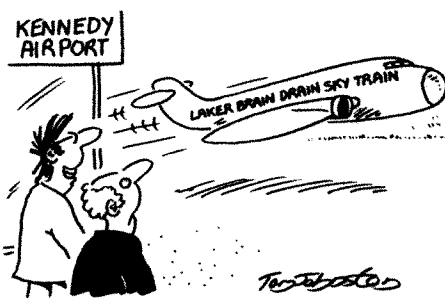
National Science Foundation, the National Institutes of Health, the University of California and Stanford University. All parties eventually agreed that NIH would enter an institutional patent agreement with Stanford University and the University of California. Stanford would administer licensing of the technology, under terms of the agreement and share royalties equally with the University of California. After public hearings last spring, NIH granted Stanford permission to go ahead with licensing, provided any company granted a license would abide by NIH safety guidelines for genetic engineering.

Niels Reimers, Stanford's manager of technology licensing says he expects a patent to be issued within a matter of months, and believes it will cover much of the basic technique for producing recombinant DNA in *E. coli*. Stanford will probably wait until the patent is actually issued to begin licensing.

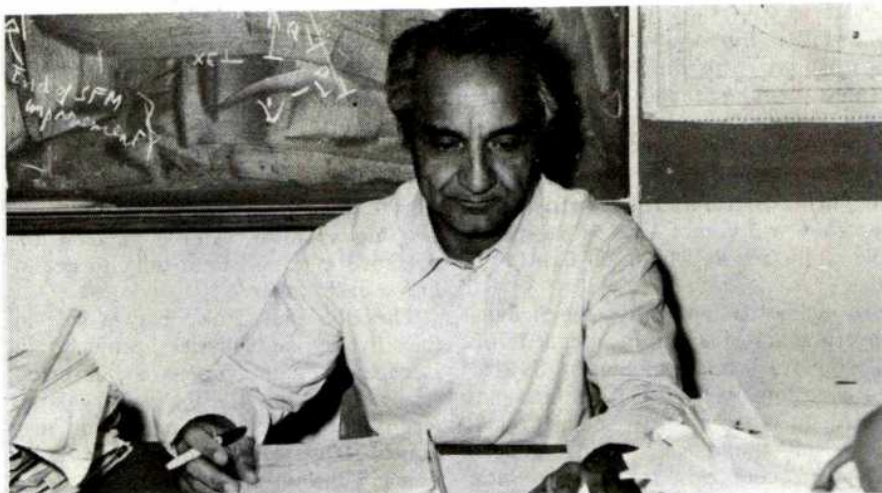
Such a patent would apply only in the United States. For that reason, and because of early stringent regulations, some scientists who are trying to create insulin by isolating and transferring a human gene have gone to Europe to work. Applications to use the country's first super-secure “P-4” facility, at Fort Dietrick, Maryland, will not be processed until later in the autumn. New, less stringent safety guidelines will also not go into effect until later this year.

William Rutter, a molecular biologist at the University of California, San Francisco, is already looking to Europe as the place to continue his work on the human gene for insulin. “One is forced to do that if one wants to do the experiments” he says. His group is planning to work in France. The potential advantage of using the natural gene for insulin, he says, is that it includes additional information for folding and joining the A and B peptide strands.

John Douglas



“Yes, we go both ways now”



Antonino Zichichi: shy of the camera, but not of politicians

Sicily's scientific evangelist

The European Physical Society, which meets next week in York, has for its president a Sicilian very powerful in Italian science politics: Professor Antonino Zichichi. He spoke recently to Robert Walgate

ANTONINO Zichichi, 48-year-old son of a Sicilian small businessman, entered university without knowing that physics could be a career. Now he is Professor of Advanced Physics at the University of Bologna. He is also virtually king of Erice, a Sicilian hill-top village where 15 years ago he established a summer school: the Ettore Majorana Centre. (A hundred scientists a week now visit Erice. "I had left Sicily. I felt guilty. I wanted to help.") Zichichi has also helped the Italian National Nuclear Physics Institute (INFN), of which he is president. Earlier this month he succeeded in doubling its budget, committing Italy to three new accelerators: an electron storage ring, a superconducting cyclotron, and an electron linac.

Professor Zichichi is a particle physicist who keeps company with the Italian ministers of science and education. And he is now President of the European Physical Society. He is also, without doubt, a thoroughgoing romantic. He is romantic about Europe. He is romantic about science. But both romances are tinged with realism: he wants to get things done.

'Culture' is an important word in his vocabulary. By it he means largely disseminated 'pure science'. Technologists, he says, are "culturally 300 years in the past; they should understand quantum electrodynamics". According to Zichichi, scientists create "new culture". "The greatest three things that mankind has created are first language, then logic, and finally science". Language needs very little logic, just enough for internal consistency. Logic,

invented 2,000 years ago, "is above language; it is something new." The most rigorous form of logic is mathematics; but you can invent anything in it, such as spaces of 25 dimensions. Science, which began with Galileo, is the logic of nature. "Science tells you if you want to believe something just ask nature. It took 2,000 years to learn that force was proportional to acceleration and not to velocity, because the Greeks only argued". Thus Zichichi believes that science is the highest achievement of mankind.

"But science has no impact in human culture. Human culture is still dominated by language. People can still be influenced by a slogan. Invent a slogan and you will have millions of people following you. A slogan is an insult to human intelligence." Science, says Zichichi, "has produced a lot of science but very little culture." What would be poetry "if poets had kept their work only in a closed drawer, or communicated only among themselves? Or painting if Raphael or Picasso had kept their paintings locked away? Or music if musicians had played only to each other? With science it's the same story. There are immense treasures of knowledge locked away in our laboratories and our libraries, but scientists have done very little to make them known to everybody."

There is a barrier to cross in that one has to work to understand science; but 300,000 years ago probably very few people had language. Using a language is not easy; but now everyone can speak. Why? Because we hear

speech from birth. "My conviction is that the same should happen with science. One day everybody will be scientists—not in the sense that they will make discoveries, but they will understand the discoveries that others are making, which is already a very great step forward".

Professor Zichichi is not unaware of the more temporal benefits of publicity. I was interviewing him at CERN, the European nuclear physics laboratory near Geneva, where he has spent most of his career since 1956. I asked him what he considered his greatest contribution to physics. It was the discovery of the antideuteron—a nucleus consisting of an antiproton and an antineutron—which he made in one night in 1965. "If you forgot the accepted wisdom about invariance principles and just thought classically, you could ask who told us that the antiproton and the antineutron stuck together like the proton and the neutron? And if they did not, the whole route to the anti-nuclei would have been stopped." Zichichi found the antideuteron. "And we didn't make any press release—nothing." Seven years later another group rediscovered it and published it widely. "At this point I decided to take more care of public impact".

Zichichi began to write for an Italian newspaper — "the greatest", *Corriere della Sera*, in a column titled 'new culture'. "I don't like to call it science popularisation". After two years he moved to *Il Giornale Nuovo*, to reach a new public, and now he writes articles of up to 1,000 words for the Saturday edition of the Rome newspaper *Il Tempo*. The latter has a circulation of 300,000, half that of his first paper, but it is read by "political people, religious people, in Rome". It is therefore more influential; and Zichichi gets more letters about his *Il Tempo* articles than any others.

If communicating science is one of Zichichi's passions, then so also are people. He sees European Physical Society first in terms of creating friendships; he sees his negotiations with ministers in terms of relationships and conversations, as a matter of convincing individuals face-to-face with his arguments. "I like to leave the administration of INFN to those who can do it better than me. I work on the scientific strategy; I try to convince people at the highest level with good arguments... Politicians are not stupid people. They are very smart. Too many of my colleagues make the mistake of thinking them stupid."

I asked Zichichi what was the place of a scientist in Italian politics: "Nonexistent."

"But you have a lot of influence." "I am the exception not the rule."

"How do you do it?"

"I don't know how I do it. I think it's because they understand me and not the others."

And yet "I don't like to waste my time with politics. I took the presidency of INFN because I thought we were going to have troubles. The first thing I like to do is the research I am interested in. But you cannot spend your life complaining . . . some time ago I was arguing about strategy and so on with my colleagues in INFN, and at a certain point they said well, you cannot refuse to be president if you really want to go on like this. I would have preferred someone else to step in; but I realised I had to accept responsibility for the things I wanted to get done."

"Do you have any other administrative ambitions?"

"No. Zero. Absolutely no." I asked about CERN. "In CERN I do only physics. I've never had any responsibility of any kind. And I'm very glad, for there must be a place where you do only physics." Could CERN be better run? He was not in a position to say. "It is always easy to criticise organisations. I have a positive attitude to anything existing. But what I can tell you is that it is easy for big organisations to slip out of purely scientific control. I am facing this problem at INFN, and that is only one-tenth the size of CERN."

"Would you like to be director of CERN?"

"No. Because if you are director of CERN you cannot do any more physics. And I want to do physics. It is all right for a theorist. But if you are an experimenter you must make a choice: either you are director of CERN or you do physics. The games I am in now, I can combine with my research; I would feel without an important part of myself if I had to give up physics."

Erice perhaps weighs equally with research in Zichichi's constitution: "I am in touch by telephone for a few minutes every day." Why did he start the summer school? For a complex of reasons. "As you know Sicily is a place of great culture. Pretty well every culture but the Chinese has been through Sicily. But it is an underdeveloped part of Europe. I was not living there, and in a certain sense I felt guilty. But what could I do?"

"One thing was clear to me. The universities started 1,000 years ago because people wanted to know what was going on at the frontier or knowledge. Books were very slow. So people had to meet. But now universities cannot bring people to the frontier because of the great development of modern knowledge. So the old university spirit has been lost."

"People still want to be brought to the frontier. But who is going to teach you? It may be that at X you have the greatest world specialist in some discipline and you are lucky; but you miss the other disciplines. So if you can establish a place where people can be brought to the frontier of various fields then they will come. That is the principle of the Ettore Majorana Centre at Erice."

The school is financed by the Sicilian Government (Sicily is independent of Italy but for foreign policy), by INFN, and occasionally by other bodies such as NATO. When it started Zichichi had offers of buildings. "But underdeveloped countries are strewn with cathedrals in the desert. I wanted to be sure this place would work. And Erice beautiful, of human dimensions."



Erice, home of Zichichi's summer school.

Erice is built on a volcanic outcrop of rock, like a natural fortress. On one street you walk on the same stones as the Romans; more recently it was like a Switzerland in Sicily, housing and protecting the richest families. Zichichi's office is the highest part of Erice. "The view from there is absolutely fantastic." The 'summer' school (it lasts from March to December) is the whole economy of the town, and, according to one enthusiastic visitor the few inhabitants (some 100 or so, says Zichichi) bow their heads to Zichichi as he passes.

Zichichi hands out prizes to the best students (in fistfuls of Lire at the end of a course, an ex-student says) "but it is not easy to win a prize. The people who go there are really very brilliant. It is a pleasure to be there."

In March this year Zichichi 'emerged' as President of the European Physical Society, through a similar process to the one that found him president of the INFN: he wanted to do things. Following in the footsteps of his predecessor—and father-in-law—Gilberto Bernardini he has a romantic ideal for the EPS, uniting East and West and forging links among nations: "We should contribute to the cultural

unification of Europe."

Zichichi also sees the EPS as a source of "one of the greatest human feelings", friendship. "In the present age when there is an enormous amount of communication and we know what is going on on the other side of the world, we don't know our neighbours two metres away. And it is very important for physicists to talk, to get to know each other."

The EPS should also play a role in the five great physics projects in Europe: a giant proton synchrotron (probably in the USSR); a very large electron-positron colliding beam machine; JET; a large synchrotron radiation laboratory; and a millimetre wave radio interferometer. It should also develop proposals on the physics components of two new teaching institutions at present under discussion in Europe: a European 'science university' and a 'Mediterranean polytechnic' for the underdeveloped regions of the Mediterranean. And it should encourage the exchange of students and professors, perhaps for whole periods of a year's teaching: "imagine the impact of a London professor of quantum mechanics teaching in say Palermo, or Leningrad, for a whole year. Sabbaticals are not enough. We need to interest governments in this."

In the end Zichichi will always come back to his physics. He is an unconventional researcher. He was the first to look at the production of leptons in collisions of hadrons, work which eventually led to a Nobel Prize for Samuel Ting in the discovery of the 'hidden charm' particle J/ψ . "Ting mentioned me in his Nobel address as the pioneer. I can be proud and ashamed—proud that I started, and ashamed that I gave up". Also he spent five years searching for a lepton heavier than the muon at Frascati, by looking at electron annihilations producing an electron, a muon, and anything—the same reaction in which Martin Perl at Stanford discovered the 1.7 GeV tau lepton ten years later (the tau was just above the Frascati energy, like the J/ψ itself. There is little doubt that Frascati's energy was very unfortunately chosen.)

Now Zichichi is "trying to break the proton". The concept that the quarks are infinitely bound into the proton "is only at the level of 'theoretical feeling'" says Zichichi. "There is no proved theorem that says they should stay in there." Zichichi, while admiring elegant theory, has no truck with theoretical speculation, any more than he has time for reading the fantasies of novelists. In the matter of the proton he wants to ask Nature her logic. He has, as he puts it, "a very realistic view".

news and views

Cholera and related diarrhoeas ('turista')

from Richard A. Finkelstein and Mary Boesman-Finkelstein

CHOLERA and the related diarrhoeal diseases extract an incalculable global toll in human misery, morbidity and mortality. They also have a direct impact on human nutrition and growth and affect world food supplies through their effect on livestock. On August 6-11 they were the subject of the 43rd Nobel Symposium* at which 30 experts discussed the epidemiology, molecular biology, immunology, pathophysiology and significance of the enterotoxigenic enteropathies as a worldwide health problem. The symposium could not have been more timely; as a result of the tremendous progress during the past decade, there was a lot to be said.

Of the many highlights of the symposium, the dramatic advances in the treatment of cholera and related diarrhoeas, the dynamic interaction between diarrhoeal disease and malnutrition, developments in understanding the pathogenesis of diarrhoea at the molecular level, and the imminence of a living attenuated vaccine for cholera and the immunologically related enterotoxigenic enteropathies are certainly worthy of further brief review.

The remarkable developments in therapy of cholera and related diarrhoeal diseases pre-eminently illustrate the practical rewards of basic research. The results would certainly have been gratifying to the late Dr Robert Allan Phillips who, were he alive, would have been a key figure at this conference, as it was he who pioneered both intravenous and oral fluid and electrolyte therapy. His observation (Phillips *Fed. Proc.* 23, 705; 1964) that the incorporation of glucose in oral solutions might completely eliminate the requirement for intravenous fluids in the average cholera case was clearly substantiated in the symposium independently by L. Mata (Costa Rica), S. C. Pal (Calcutta), D. Barua (WHO, Geneva), W. B. Greenough, III (Dacca), T. Hendrix (Johns Hopkins), M. Field (Chicago), and M. Levine (Maryland). All but a small percentage of diarrhoeal patients can now be treated successfully solely

with orally administered electrolyte solutions. This can be accomplished, effectively and economically, by the distribution of packets containing preweighed amounts of glucose, NaCl, KCl and NaHCO₃ to be added to measured volumes of water in the hospital, in local treatment centres and, most importantly, in the home, even in the most rural and underdeveloped areas. In a small number of severe cases, particularly those with emesis precluding oral rehydration, intravenous fluid therapy is still required, but even some of these can be treated orally if the electrolyte solutions are administered in small but frequent increments. It is only a minor limitation that antibiotics, which have been shown to reduce the duration of bacterial shedding and of the diarrhoea, are not presently included. The impact that decentralisation of treatment might have on the ecology of the pathogens involved should be a subject of surveillance in the future. Other questions that remain to be answered are whether other energy sources can be substituted for glucose and whether different formulae are required for diarrhoea due to rotaviruses or other agents.

That certain antibiotics, administered prophylactically, can reduce the incidence of diarrhoea in travellers was established by B. Sack (Johns Hopkins University) in studies with Peace Corps volunteers. In this population, enterotoxigenic *Escherichia coli* are responsible for the majority of cases and, fortuitously at present, the majority of these strains are antibiotic sensitive. However, because of the spectres of drug resistance and alterations of normal flora leading to superinfection, participants at the conference agreed that prophylactic antibiotics, if used at all, should be restricted to short-term travellers at high risk.

The possibility of pharmacological intervention after the onset of diarrhoea was also raised and supported by experimental studies by Hendrix and I. Lönnroth (Göteborg). At the moment, two drugs, chlorpromazine and nicotinic acid, show promise in animal studies and merit further consideration for controlled study in man.

Contrary to popular belief, evidence presented by Mata indicates that rather

than malnutrition predisposing to infection, the reverse seems to be the rule—'malnutrition is determined by diarrhoea.' With newborn infants and children in particular, especially in developing areas, there is a tendency to withhold nutrients during diarrhoeal illness. Mata's studies in Central America, supported by observations of Barua, clearly indicate that when such children are adequately treated, by oral therapy, during their multiple bouts of diarrhoea, they grow better than their counterparts—the stunting which occurs during and following illness does not happen. This work has the important implication that availability of food is not the major problem in 'malnourished' children. Rather, the problem is to educate the people to provide proper treatment and normal (adequate) diet throughout periods of diarrhoeal disease. This is particularly important during the nursing period. Mata's message was also that we should be careful to differentiate between susceptibility and severity, recognising that the malnourished individuals are most frequently found in poor sanitary environments.

Epidemiologically, it was acknowledged that cholera declines when the water supply is freed of contamination with human faeces. It is not clear, however, that this is the case with other enterotoxigenic enteropathies such as *E. coli* diarrhoea, which seems to be transmitted primarily within the household and by food. The problems of developments in sanitation, which could solve the cholera problem, while recognised by the participants, were felt to be beyond the province of this conference.

Presentations by M. Gill (Harvard University) and M. Field (University of Chicago) revealed that for the second and third times (diphtheria being the first), the pathogenesis of infectious diseases is within a hair's breadth of being totally understood at the molecular level. Field, who inspired the recognition that cholera enterotoxin (choleragen) acts by activating host cell

*The symposium was held at the Nordic Education Centre, Stockholm, and was excellently organised by Orjan Ouchterlony, Jan Holmgren, John Craig and Dhanan Barua. Review papers provided the foundations for workshops culminating in recommendations for the future. The complete proceedings, workshop summaries and recommendations are to be published by S. Karger, AG, Basel, early in 1979.

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adenylate cyclase, presented new evidence that ST (the heat-stable low molecular weight enterotoxin(s) of *E. coli*, of which one example was characterised by D. Robertson (University of Kansas) as a peptide of 47 residues) acts by activating host cell (specifically intestinal epithelium) guanylate cyclase, while Gill indicated that the A-subunit of cholera toxin catalyses primarily the ADP-ribosylation of a GTP-binding protein which, according to observations by Z. Selinger, traps adenylate cyclase in its activated state. The subsequent events, catalysed by cyclic-AMP or cyclicGMP, which lead to the increased electrolyte and fluid secretion, are still a grey area.

S. Falkow (University of Washington, Seattle) dynamically demonstrated the application of the newly-developing recombinant DNA technology to the solution of problems of pathogenesis, with the object of creating useful new microbial strains, rather than new pathogens, with evidence for the cloning of the ST and LT (heat-labile cholera-related enterotoxin) genes of *E. coli*. He supported the observations of F. Ørskov (University of Copenhagen) that the *E. coli* strains which are selected to become enteropathogens (that is, which can effectively receive *tox* plasmids and other essential virulence factors) may be restricted to a relatively small number of serotypes which happen to be relatively good genetic recipients. Some 'non-toxigenic' strains of *E. coli* of proven pathogenicity for man in volunteer studies may produce toxins which are active in other than the usual models, according to Levine. Other essential attributes of virulence, such as chemotaxis and adhesiveness leading to colonisation, were discussed by R. Freter (Michigan). J. Craig (New York) pointed out that cholera vibrios differ in the amount of toxin they elaborate *in vitro* under two different conditions of cultivation. The advantages and limitations of available techniques of recognising enterotoxins and their antibodies were discussed by Ö. Ouchterlony, who also presented a novel simple method of assay based upon changes in hydrophilic character of antigen-antibody complexes on plastic surfaces. The essential roles of the host cell glycolipid receptors, the G_{M1} ganglioside, elucidated by the pioneering observations of W. E. van Heyningen, were reviewed and extended by L. Svennerholm and J. Holmgren (Göteborg).

The possibilities of immunological intervention were high among the priorities of the Symposium. J. Feeley (Atlanta) extensively reviewed the scientifically controlled field studies of cholera vaccines composed of killed vibrios administered parenterally, and concluded, in accord with previous

judgment, that the benefits derived did not justify the cost of vaccination. According to Barua, in consideration of these observations, requirements for cholera vaccination were removed from WHO regulations in 1973. Nevertheless there is the possibility of improvement, as brought out by more recent studies, presented by I. Joó (Hungary) and Pal, on alum-adsorbed bacterial vaccines which seem to generate greater protection in the susceptible infants and children of endemic areas than previously tested un-adsorbed vaccines. Although cholera toxoid administered parenterally offers no protection according to the field studies summarised by N. Ohtomo (Japan) and G. Curlin (Dacca), the synergistic protective effect observed experimentally with combinations of toxin antigen and somatic antigens in studies by A.-M. Svennerholm (Göteborg) is worth further consideration. She also indicated the potential value of assaying the antibody content of milk from immunised lactating women as a reflection of the local (intestinal) secretory antibody response. N. Pierce (Johns Hopkins University) reviewed extensively his observations on the kinetics of formation of antibody-containing cells in the lamina propria in response to a variety of combinations of orally and parenterally administered toxin antigens in experimental animal models and concluded that the nature of the antigen is important and that immunological suppression may result from some regimens. J. Holmgren (Göteborg) indicated that species differences may also be important, as illustrated by his studies in the orally immunised mouse model described earlier by Fujita and Finkelstein. J. Murphy (Harvard) reviewed genetic studies in his laboratory which have resulted so far in the isolation of two hypotoxinogenic mutants of strain 569B which produce reduced amounts of subunit A and little, if any, subunit B.

That solid immunity against cholera is indeed feasible has been established conclusively by studies in volunteers as summarised by Levine. Volunteers convalescent from induced cholera were found to be virtually completely refractory to subsequent challenge with cholera vibrios of either the homologous or heterologous serotype. Therefore it is clear that the disease itself is an immunising process, although the mechanism(s) and the nature of the immunising antigen(s)—somatic antigens, enterotoxin, colonisation factors, flagella, outer membrane proteins, or a combination—are not yet known. Immunity to rechallenge after induced *E. coli* diarrhoea was markedly less effective.

In an earlier session, R. A. Finkelstein (University of Texas, Dallas) sum-

marised the laboratory production and isolation of enterotoxins; elucidated criteria for defining enterotoxins and 'enterocytotoxins'; issued a caveat against the tendency to regard all newly described toxicities as toxins; and also reported the isolation with T. Honda, of a mutant of *Vibrio cholerae* which offers promise as a live vaccine against cholera and related diarrhoeas. Discussed further in the Immunology workshop, the mutant, called 'Texas Star', is avirulent in the sensitive infant rabbit model, has shown no signs of reversion, and seems to produce only the immunogenic B (binding) portion of the cholera enterotoxin. It is hoped that the mutant will be shown to be innocuous in volunteer studies and that it will simulate infection with virulent vibrios by producing solid immunity to cholera, and potentially to other diarrhoeas as well, by means of the common toxin B antigen. As a previous hypotoxinogenic mutant, M13, isolated in our laboratory, was harmless in volunteers and induced cholera immunity, this expectation may not be unrealistic.

In the immunology workshop it was emphasised that in volunteer studies, which are an effective step toward vaccine development, the highest internationally acceptable ethical standards must be followed with regard to selection, education, and respect for the rights of volunteers.

Participants were reminded of how far we have come in a short time by the presence of one of the Indian investigators, S. N. De (Calcutta), who in the late 1950s first showed that the symptoms of cholera could be produced in laboratory models by cell-free products of the cholera vibrio. □

Ultra-cold neutrons in superfluid helium

from P. V. E. McClintock

THE first measurements of the rate at which ultra-cold neutrons are produced within a vessel of liquid helium, reported in a recent issue of Physics Letters (66A, 469; 1978), strongly support the earlier suggestion by Golub and Pendlebury (Physics Letters 62A, 337; 1977) that it might be possible to fill a neutron bottle with trapped neutrons to an unprecedentedly high density if superfluid ⁴He were used as the internal medium. The production measurements were carried out in Grenoble at the Institut Laue-Langevin by P. Ageron and W. Mampe (ILL) in collaboration with R. Golub and J. M. Pendlebury (Sussex University).

Ultra-cold neutrons (UCN) are neu-

trons of such low energy that they are totally reflected from material surfaces at all angles of incidence, so that it becomes possible to confine them inside a suitably designed container (neutron bottle), where experiments can then be conducted in a relatively leisurely fashion—compared to neutron beam experiments where the time of observation is restricted to about 1/30 s at most—to elucidate their properties. If they could be trapped at sufficient density for periods comparable with their lifetime (neutrons decay by β -emission in about 15 minutes), then it might become possible to determine certain fundamental constants, such as the electric dipole moment of the neutron for which there are various theoretical predictions but, as yet, no positive measurements. The main difficulty is that the present technology available for UCN production, exploiting the very low energy Maxwellian tail of the thermal distribution of neutrons within a nuclear reactor, leads to trapped UCN densities of only about 1 cm^{-3} , which is not really adequate for the proposed experiments.

Golub and Pendlebury's suggestion amounted to a completely different technique by which UCN might be produced: by down-scattering in liquid helium of neutrons with an initial velocity of about 450 m s^{-1} . They pointed out that, when a neutron of this particular velocity travels through a bath of superfluid ^4He , there is a finite probability that it may spontaneously create a thermal excitation (in effect, a single quantum of sound) and, in doing so, lose almost all its initial energy. In its final state it will be an ultra-cold neutron, moving at less than 5 m s^{-1} , and unable to escape from the container. Their proposed UCN source consists, therefore, of a container of superfluid ^4He whose walls are transparent to neutrons with velocity of 450 m s^{-1} , placed in a beam containing a relatively large proportion of neutrons of this velocity. Most of the neutrons will pass straight through and out the other side, but a certain number will create excitations, enter the UCN region and become trapped.

The UCN density attainable by this method clearly depends on a balance between the production rate and the loss rate. Losses of UCN from the bottle will occur inevitably through β -decay but, in addition, can also arise as the result of up-scattering by thermal excitations (the converse of the production mechanism), absorption by impurities in the ^4He , or inelastic scattering from the walls. The latter phenomenon has restricted the storage times achieved in evacuated room-temperature bottles to about 30 s, but it is expected to become much less important at the temperatures over

1 K where the helium filled bottle will be operated. The most potent neutron absorber likely to be present in the liquid is the other isotope of helium, ^3He : it will be necessary to reduce the ^3He isotopic impurity content to a level about four or five orders of magnitude below that (a few parts in 10^7) found in ordinary commercial ^4He . Fortunately, however, purities even greater than this can quite readily be achieved using the heat-flush isotopic separation method recently developed at Lancaster, and neutron absorption within the body of the liquid is not therefore expected to be a problem. Neither should up-scattering processes since, provided the bottle is operated at a temperature below 1 K, the number of excitations with the appropriate energy to initiate such events will be quite negligible. The main unknown factor has been the production rate and, although Golub and Pendlebury did their best to estimate it, the calculation was by no means straightforward and it incorporated a number of assumptions which could have been in error. Hence the importance of the work now being reported from the ILL, which constitutes the first experimental measurement of the UCN production rate in superfluid helium.

The arrangement used was extremely simple. A vessel of liquid ^4He , whose temperature could be reduced by pumping, was placed in a horizontal neutron beam. Ordinary commercial ^4He was used, and so no provision was made for storing any UCN which might be produced. Instead, a neutron guide tube was provided to lead the UCN out of the liquid helium to an external neutron detector. The guide incorporated five right-angled bends to enable neutrons of higher energy to escape through its walls and thus to ensure that only the UCN could reach the detector and be counted.

In the event, the neutron counting rate increased rapidly to about 5 s^{-1} when the temperature of the liquid was reduced below its superfluid transition temperature at 2.2 K. When a 0.5 mm thick stainless steel plate, totally opaque to UCN but partially transparent to neutrons of higher energy, was placed so as to block the guide tube, the counting rate fell to the background level of 0.5 s^{-1} ; but it returned to its former value again as soon as the plate was removed. Some 24 hours later, as the last of the liquid helium boiled away, the rate fell once more to the background level. It seems virtually certain, therefore, that the detected neutrons were UCN produced in the liquid through the mechanism proposed by Golub and Pendlebury. Furthermore, the measured production rate was found to be in remarkably good

agreement with the value which they had calculated.

This is a very encouraging result. It implies that the fullscale neutron confinement cryostat now being planned at the ILL, which will incorporate a sample of isotopically ultra-pure liquid ^4He , is likely to store UCN at densities at least sixty times larger than have been attained by conventional methods—and perhaps a thousand times larger, if the problem of wall losses can be overcome. □

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Progress in Fusion Research

from a Correspondent

TOKAMAK rules. That was the essential message from the 7th International Conference on Plasma Physics and Controlled Nuclear Fusion Research held at Innsbruck from 23–30 August last. The results from the large (PLT) tokamak at Princeton (USA) were presented and discussed in some detail. In particular the scientific basis for the widely publicised claim that a temperature of 60 million degrees had been reached was carefully examined and finally accepted. The discussions highlighted the difficulties of unambiguous measurement of ion temperatures in such plasmas. Indeed it can be argued that the obsession with 'temperature' is a hangover from the Zeta days and that in these plasmas, particularly those heated by powerful neutral beams, the single crucial number is the ratio Q of the energy released in fusion reactions to the energy input. In the Princeton experiment with 1.7 MW of neutral deuterium beam power into a deuterium plasma, a neutron emission of $4 \times 10^{12}\text{ neutrons s}^{-1}$ was measured; at $\sim 3\text{ MeV}$ per reaction, one can calculate that this corresponds to $\sim 20\text{ W}$ of fusion power and hence to $Q \sim 10^{-4}$. If a tritium beam had been used, then the more favourable cross-section and larger energy release would have increased Q to $\sim 5 \times 10^{-3}$. The new tokamak TFTR at present under construction at Princeton is designed to achieve $Q > 1$ through the reduction in diffusive losses by the use of larger dimensions. In the light of the new results its chances of success look good. For a power reactor $Q \sim 20$ would be needed.

Results from other tokamak experiments around the world showed an increasing ability to reduce the contamination of the plasma by impurities. With cleaner plasmas it is possible to reach higher densities than before and to increase the ratio of toroidal plasma current to toroidal magnetic field. Thus

in the Alcator experiment a record value of the product of plasma density and containment time was reached, ie. $\langle n\tau \rangle = 3 \times 10^{13} \text{ cm}^{-3} \text{ s}$. The plasma here was relatively cold with a temperature of ~ 8 million degrees.

Despite the success of tokamaks in confining hot plasma the difficulties of developing the concept into a practical power producing reactor are immense. One major problem is the relatively low ratio β between the plasma pressure and that of the magnetic field. The plasma produces the power while the magnetic field costs money. Thus many studies have suggested that the minimum acceptable β for a reactor is 5–10%. In present tokamaks β is typically less than 1%. Theoretical work from Culham, Lausanne and Princeton presented at the conference suggests that the maximum β in a tokamak consistent with plasma stability is only about 3%. This leaves an awkward gap and it is therefore of crucial importance to carry out definitive experiments to test the theory.

The fact that the road to the tokamak reactor is clearly a very difficult one encourages the continuation of work on other magnetic confinement approaches where the difficulties are equally daunting but different.

Thus work continues on a variety of magnetic confinement schemes including the stellarator, reversed field pinch and mirror machine. Steady progress on these lines was reported at Innsbruck but no startling breakthrough of the kind that would lead to any switch of emphasis away from the tokamak. A dark horse in the competition is the Elmo Bumpy Torus (EBT) experiment at Oak Ridge. In this experiment a steady state toroidal plasma is produced by high power (60 kW) microwave heating with an ion temperature of about 2 million degrees. The practicability of this approach has recently been greatly increased by technical developments in the generation of high power millimetre microwaves. A reactor design based on the EBT principle was presented at Innsbruck. A microwave power of the order of 100 MW at $\sim 100 \text{ GHz}$ is envisaged.

The major alternative to magnetic confinement is the inertial approach in which a tiny pellet is compressed to densities of $\sim 10^{25} \text{ particles cm}^{-3}$, heated to thermonuclear temperatures and holds together under inertial forces for long enough (10^{-11} s) to give an adequate yield of fusion energy. Presentations on this subject at Innsbruck were relatively muted, the major groups being mainly pre-occupied with getting the necessary multi-terrawatt driver systems (lasers, ion beams and electron beams) to work. Thus the essential

feature of compression to very high densities has yet to be experimentally demonstrated. Most existing experiments are done with so called exploding pusher targets. In these high pressure (100 atm) deuterium and tritium gas is compressed by the explosion of the surrounding glass microsphere. Experiments of this type around the world with CO_2 and Nd glass lasers (including one at the Rutherford Laboratory), have produced a great deal of information, diagnostic experience and Q values of $\sim 10^{-3}$. Experiments aimed at $Q \sim 1$ using the Shiva laser at the Lawrence Livermore Laboratory (Nd glass, 26 TW) and the Antares laser at Los

Alamos (CO_2 , 100 TW) are due to begin soon. The problems of converting these inertial confinement systems into fusion reactors with the necessary high repetition rate and adequate containment of the successive small explosions are very great. The Livermore group propose to surround the exploding targets with a 100 ton, 1 m thick falling curtain of liquid lithium to absorb the neutrons and reduce radiation damage to the external structure.

Certainly all the concepts presented at Innsbruck lead to very complex and difficult reactors. The assessment and mastery of this complexity will be one of the major tasks for the coming years. $\square$

Zr₃Al creeps in a petty pace

from Robert W. Cahn

In 1956, A. H. Cottrell predicted that metallic uranium, while undergoing steady fission, would lose most of its resistance to creep under stress, and he initiated a very simple experiment with a loaded uranium spring inside a reactor. His hypothesis of enhanced irradiation-creep was promptly confirmed and this had immediate consequences for the design of the British nuclear reactors of that time.

Cottrell's crucial hunch has been followed up in the intervening years by a large body of experiment and theory. Not only a fuel but also its container is subject to this form of creep, and it is a matter of special concern when a 'cladding material' is weakened, for the container has the function of retaining fission products both solid and gaseous; the latter exert a pressure which must be resisted by the container. After much experiment as well as very sophisticated theoretical physics during the past few years, the mechanisms both of irradiation-creep and of the related process of void-swelling (when minute cavities form in the container during reactor service) are now understood in outline. Neutron irradiation produces equal numbers of vacancies and interstitials, some of which recombine quickly; the rest migrate to and become absorbed at 'sinks', of which dislocations are the most important. The crucial point is that a dislocation is a 'biased sink'—that is to say, it has a slightly greater tendency to absorb interstitials than vacancies (the bias is typically around 2%); this bias is explicable in terms of differing elastic interactions of the dislocation with the two types of point defect. The excess of vacancies so generated eventually condenses to form the minute aggregates called voids (whose existence was first reported in *Nature* in 1966). Furthermore, an external stress can increase the bias and thus

accelerate the climb of dislocations which must result from a net flux of interstitials to dislocations. That accelerated climb in turn is apt to produce accelerated creep under the action of the external stress, since under certain circumstances, the climb rate is rate-determining for creep.

The recognition of the central importance of point defect kinetics in irradiated alloys has opened the way for a new kind of alloy design—the attempt to predict what kind of alloy might be least susceptible to enhanced creep and void-swelling. The matter is of special interest in fast reactors and in water reactors; the latter all use container materials based on metallic zirconium, which combines an appropriately low neutron capture cross section with good corrosion resistance in hot water under irradiation. A determined attempt is now under way in Canada to improve on the 'zircalloys' in current use, and the project is based on a most ingenious hypothesis—the postulated influence of atomic order in an alloy, Zr_3Al , in reducing the net flux of interstitials to dislocations and, thus, in reducing liability to irradiation-creep.

It is well established from comparative studies of successful and unsuccessful industrial innovations that the most important single factor in ensuring success is a resolute and energetic 'product champion'. The champion of Zr_3Al is E. M. Schulson, a metallurgist on the staff of Chalk River Nuclear Laboratories in Canada. He argued in 1972 that this alloy had the virtues of low neutron absorption and promising mechanical properties, and further suggested that irradiation-creep might be reduced. The basic postulate here is that ordering generally reduces the rate of vacancy migration in an alloy (there is an often cited study of beta-brass, in 1956, which first estab-

lished this point); at any given temperature, a slowing down of vacancy migration gives more time for recombination of vacancies and interstitials and this reduces the net flow of interstitials to biased sinks, and thereby reduces irradiation-creep rate under a given stress. In pursuit of this hypothesis, Schulson has orchestrated a large range of studies, most of which have come to publication during the past 1½ years: it now looks as though his ingenious hypothesis is justified, though a full study of the irradiation-creep itself is yet to appear.

The hypothesis itself is outlined in a paper by Schulson in *J. Nucl. Mat.* **66**, 322; 1977. Here he also reports that stress-relaxation of Zr_3Al under neutron or 1 MeV electron irradiation at 570 K (a realistic reactor temperature) is a factor of three or more lower than that for any zirconium-based alloy studied to date. This striking experiment is to be fully described in a conference report to be published by ASTM. Zr_3Al is ordered (Cu_3Au type, $L1_2$) up to its peritectoid transformation at 1,265 K, but can be progressively disordered by radiation damage. Several studies have sought to establish whether, at temperatures of practical interest, radiation-disordering goes far enough to nullify the advantage which forms the basis of Schulson's hypothesis. This is a matter of balance between radiation damage and spontaneous reordering, and this balance depends on the rate of damage and the temperature. Accelerated damage by ion bombardment (Howe & Rainville *J. Nucl. Mat.* **68**, 215; 1977) or 1 MeV electron bombardment (Carpenter & Schulson *J. Nucl. Mat.* **73**, 180; 1978) showed that at realistic service temperatures, large doses leading to several displacements per atom were required to produce substantial disorder above 575 K. Neutron bombardment in reactors would produce less damage and therefore less disorder than either ions or 1 MeV electrons. Further studies (Rosinger *J. Nucl. Mat.* **66**, 193; 1977; Schulson, *Acta Met.* **26**, 1189; 1978) have established that neutron irradiation strengthens Zr_3Al at all temperatures, and another (Schulson & Roy *J. Nucl. Mat.* **71**, 124; 1977) has elicited the remarkable fact that irradiation actually reduces the notch-sensitivity of the alloy (which is in any case not severe) and, at room temperature, the notched tensile strength is even enhanced by irradiation. This behaviour is quite at variance with the normal effect of irradiation on notch-brittleness, which is pronounced, for instance, in ferritic steels. Another study (Schulson *Met. Trans.* **9A**, 527; 1978) established that the fracture morphology of Zr_3Al indicates a ductile fracture mechanism.

These various experimental studies have now been supplemented by a thorough theoretical investigation of the kinetics of point defect annihilation and irradiation creep for a number of idealised imaginary alloys with various types of atomic order (Schulson, Swanson & MacEwen *Phil. Mag.* **A37**, 575; 1978). This very thorough analysis is based on certain assumptions about the change of migration energy for vacancies as a result of ordering and the further assumption that sink strengths are not affected by ordering. Numerical results are obtained for vacancy concentration as a function of temperature, for net interstitial flux to dislocations for various assumed vacancy migration energies and, finally, for the factor of reduction of dislocation climb rate due to ordering. In the temperature range of interest, for the

idealised $L1_2$ alloy, the climb rate (and therefore the irradiation-creep rate) is shown to be reduced by 30–70%, depending on dislocation density and whether simple or super-dislocations are present. This paper marks a milestone in the application of exact atomistic theory to the prediction of irradiation behaviour.

Zr_3Al may turn out to behave even better than theory indicates, because preliminary electron microscopy indicates that the alloy, after irradiation, has a smaller than normal density of dislocation loops. Some unidentified healing mechanism, it seems, may render the alloy even less liable to the processes that enhance irradiation-creep than is predicted by theory.

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Unfolding Troponin-C

from Jonathan Seymour

TROPONIN-C (TN-C) is one of the most interesting proteins in muscle, because it lies at the kernel of the mechanism for controlling contraction and relaxation. When the concentration of Ca^{2+} around the thick and thin filaments of muscle is high, TN-C binds Ca^{2+} and contraction takes place. In contrast, when the Ca^{2+} concentration is low, TN-C loses its bound Ca^{2+} and contraction is inhibited and the muscle relaxes. This inhibition of contraction is produced by tropomyosin and the troponin complex, both of which are situated on the muscle thin filaments. The troponin complex consists of three components: TN-I, which exerts the inhibitory action; TN-T, which binds the complex to tropomyosin and TN-C, which is responsible for binding Ca^{2+} . Recent experiments have revealed how the structure and functions of TN-C are related.

TN-C has two binding sites which can bind either Ca^{2+} or Mg^{2+} . At low concentrations of Mg^{2+} , these Ca^{2+} - Mg^{2+} sites show a high affinity for Ca^{2+} . Also TN-C has two further sites that can bind Ca^{2+} only. These Ca^{2+} -specific sites show a lower affinity for Ca^{2+} than the Ca^{2+} - Mg^{2+} sites, in the absence of Mg^{2+} . The application of such techniques as proton magnetic resonance, circular dichroism and a measurement of tyrosine fluorescence have revealed the effects of Ca^{2+} binding at the different sites of TN-C. When Ca^{2+} binds to the Ca^{2+} - Mg^{2+} sites, the backbone of the polypeptide chain refolds, generating new lengths of α -helix. This large re-organisation of the molecule results in TN-C adopting a more stable conformation. Similar, but not identical, changes occur when Mg^{2+} is bound to the Ca^{2+} - Mg^{2+} sites. Further binding of Ca^{2+} at the Ca^{2+} -

specific sites results in smaller changes occurring, but there is some evidence for an accompanying increase in α -helix.

The primary sequence of TN-C reveals four regions (I to IV) of the polypeptide chain that are homologous with the Ca^{2+} binding sites of parvalbumin. Parvalbumin is a Ca^{2+} binding protein whose three dimensional structure has been solved, to an atomic resolution, by X-ray crystallography. These Ca^{2+} binding sites consist of a bend in the polypeptide chain, rich in acidic side chains, and flanked on both sides by α -helical regions. The immediate question arises, which regions of the sequence (I to IV) correspond to Ca^{2+} - Mg^{2+} sites and which to Ca^{2+} -specific sites? Detailed analyses of the sequences in the four regions led to contradictory results. Further progress was made by attaching probes to one cysteine residue in region III of the molecule. Using fluorescence measurements and E. S. R. techniques, it was established by Potter *et al.* (*J. of Biol. Chem.*, **251**, 7551; 1976) that region III corresponded to a Ca^{2+} - Mg^{2+} site.

Recently, the nature of all four regions has been unambiguously resolved by studying the Ca^{2+} binding properties of fragments of TN-C. Leavis *et al.* (*J. of Biol. Chem.*, **253**, 5452; 1978) used trypsin, thrombin and cyanogen bromide to cleave TN-C at different points along its polypeptide chain. The resulting fragments contained one or more of the four binding regions (I to IV). It was shown that regions III and IV corresponded to the Ca^{2+} - Mg^{2+} high affinity sites, in agreement with the earlier assignment of region III. It follows immediately that regions I and II correspond to the Ca^{2+} -specific sites. In addition two regions

of TN-C were identified that bound to TN-I. This is in agreement with similar work done on TN-C fragments by Weeks and Perry (*Biochem. J.*, 173, 449; 1978) and also with earlier work done on TN-I.

At the same time the work of Leavis *et al.* revealed a limitation of looking at fragments of TN-C. It was found that fragments containing the two Ca^{2+} - Mg^{2+} sites, regions III and IV, had a strong affinity for Ca^{2+} and could also bind Mg^{2+} . Further, cleaving the polypeptide, so that regions III and IV were on separate fragments, resulted in the isolated Ca^{2+} - Mg^{2+} binding sites changing their characteristics. Now they could only bind Ca^{2+} weakly and failed to bind any Mg^{2+} at all. This illustrates the importance of tertiary structure (relationships between different regions of the same molecule) for defining the properties of the binding sites. Indeed, the Ca^{2+} -binding properties of TN-C are related to its quaternary structure (relations to surrounding protein molecules), because when TN-C binds to TN-I, its affinity for Ca^{2+} increases by an order of magnitude.

In order to obtain a better understanding of how Ca^{2+} binding to TN-C releases the inhibitory effect of the troponin complex and tropomyosin, it is crucial to know which Ca^{2+} binding sites on TN-C are the regulatory ones. The bulk of the evidence suggests that it is the Ca^{2+} -specific sites that regulate contraction. Work with myofibril preparations indicate that the Ca^{2+} concentration at which their ATPase rate is activated is independent of the concentration of Mg^{2+} . Hence, only the Ca^{2+} -specific sites are involved in regulation; but work on the tension development in muscle suggests that raising the concentration of Mg^{2+} increases the Ca^{2+} concentration required for the muscle to be activated. This possibly implicates the Ca^{2+} - Mg^{2+} sites in regulation.

The solution of this dilemma would be an important advance in understanding how TN-C regulates contraction. A crucial piece of data relevant to this question is the concentration of Mg^{2+} in muscle. This is not accurately known, but it is believed to be in the range 0.1 to 5 mM. This knowledge would enable the metal ions bound to TN-C to be identified in contracting and relaxed muscle. When the Ca^{2+} concentration is high and the muscle is contracting, all four sites on TN-C bind Ca^{2+} . However in relaxed muscle when the Ca^{2+} concentration is low, the Ca^{2+} -specific sites are free of Ca^{2+} , while the Ca^{2+} - Mg^{2+} sites could be binding Ca^{2+} or Mg^{2+} , depending upon the concentration of Mg^{2+} . There is also much more to learn about the relationships between TN-C and its

neighbours TN-I and TN-T. For it is these changing relationships, as TN-C binds and releases Ca^{2+} , that release and trigger the inhibitory action of troponin and tropomyosin.

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Nuclear resonance & synchrotron radiation

from G. V. Marr

THE electromagnetic radiation (synchrotron radiation) emitted by high energy electrons when they are constrained by magnetic fields to move in circular orbits in an electron synchrotron or storage ring is now well known and much used as an intense source of ultraviolet and X-ray photons. Because the electrons are injected as localised bunches into the synchrotron and are accelerated to speeds approaching the speed of light, the synchrotron radiation emitted is highly polarised and concentrated into tightly collimated beams forward-directed along tangents to the instantaneous electron orbit. The radiation emitted is characterised by the electron energy and the bending radius of the orbit so that its spatial and energy distribution is calculable. It appears as a continuum of radiation extending from the infra-red to the X-ray region with a peak in the XUV or X-ray region. It also possesses a pulsed-time structure determined by the orbiting frequency of the electron bunches. There are now more than 20 laboratories throughout the world, 7 of which are in Western Europe, where facilities exist for the exploitation of synchrotron radiation and research programmes are in operation to study problems in atomic, molecular, surface and solid state physics, in chemistry, and in the structural properties of solids and biological samples. In addition, X-ray microscopy, lithography, the calibration of light sources and detectors, etc., are applications of synchrotron radiation.

New applications are continuously appearing and an interesting extension of these activities to the observation of nuclear resonance excitation has been reported by R. L. Cohen *et al.* (*Phys. Rev. Letters* 41, 381; 1978). The interest is not so much that new data on the nucleus has been revealed, indeed the observations are of the well known 14 keV level of ^{57}Fe , but that the apparatus developed enables the resonance to be detected by the observation of a weak flux of conversion electrons emitted by the decay of the nuclear state in the presence of a much more intense electron flux from non-

nuclear electronic processes.

The method makes use of the fact that the University of Stanford storage ring can provide pulses of synchrotron radiation 0.3 ns wide every 780 ns. With a long lifetime (100 ns) against decay of the nuclear level it has been possible to gate the detector system so as to collect data 100 ns following the incidence of the synchrotron radiation pulses, thereby allowing photoelectrons, Compton electrons and long lived secondary electrons to dissipate before the detector is activated.

The synchrotron radiation was dispersed by a crystal X-ray monochromator to provide a band width of 3 eV photon energy centred about the 14 keV excitation energy. This radiation fell onto a ^{57}Fe enriched iron foil and the emitted electrons entered a continuous semi-conducting dynode electron multiplier which was divided into two stages with a gated grid between the two sections. The grid was gated "off" during the prompt photoelectron pulse and so removed the main cause of afterpulsing electrons which would be otherwise indistinguishable from the nuclear conversion electrons. Very low energy electrons having flight times of as long as 100 ns were removed at the electron multiplier entrance.

Under these conditions, the crystal monochromator was scanned over the energy of the ^{57}Fe nuclear resonance and the counting rate plotted as a function of X-ray energy. The width of the resonance is negligible (5×10^{-7} eV) compared with the band width of the monochromator so that the scan actually profiled the transmission function of the monochromator and the width and shape of the response reflect the monochromator output. Nevertheless the resonance was observed to occur at exactly the anticipated energy and was of the correct intensity. The authors also point out that their detector system can be used with advantage to provide a study of the Bragg-scattering profile of crystal monochromators in synchrotron radiation beam lines, thus allowing maladjustment of the monochromator or defining slits to be corrected *in situ*.

New synchrotron radiation sources are being designed specifically to make use of the synchrotron radiation rather than to provide high energy particles. In particular, the new 2 GeV storage ring under construction at the Daresbury Laboratory in the U.K. will be used to study the time structure of the radiation. Looking further ahead, the European Science Foundation is currently considering the design requirements for a European Synchrotron Radiation Facility for the 1980s.

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review article

Fibronectins—adhesive glycoproteins of cell surface and blood

Kenneth M. Yamada & Kenneth Olden*

A recently characterised class of adhesive, high molecular weight glycoproteins is present on the surfaces of cells, in connective tissue matrices, and in extracellular fluids. These proteins may have important roles in cellular adhesion, malignant transformation, reticuloendothelial system function, and embryonic differentiation.

RECENT attempts to unravel the molecular mechanisms of cellular adhesion, malignancy, hemostasis, host defense by the reticuloendothelial system, and connective tissue structure have implicated glycoproteins with subunit molecular weights of 200,000–250,000. These proteins are now thought to be closely related, and are termed fibronectins (from the Latin *fibra*, fibre, and *nectere*, to bind, tie)¹. We shall review the discovery, structure, and possible biological roles of these glycoproteins (for related reviews, see refs 2–6).

Discovery and nomenclature

In 1948, Morrison *et al.*⁷ isolated a partially purified fraction of human plasma which they termed 'cold-insoluble globulin'. Other workers independently described the various proteins or factors listed in Table 1, which were named according to sources or biological activities (Table 2). Recent evidence indicates that all of these proteins are closely related, and that they are probably only one or two specific proteins—cell surface (cellular) fibronectin, and plasma fibronectin. Cell surface fibronectin is a major constituent of the cell surface of many cultured cells, and was discovered when cell surface proteins or carbohydrates were labelled radioisotopically or immunologically (Tables 1 and 3). This glycoprotein is also known as LETS protein¹⁹ or CSP²⁰, and has an apparent subunit molecular weight of between 200,000 and 250,000 by SDS-polyacrylamide gel electrophoresis^{2–6}. Plasma fibronectin is a dimeric glycoprotein with subunit polypeptides of 200,000–220,000 that circulates in vertebrate blood^{7,23–26}. Although also known as cold-insoluble globulin, purified plasma fibronectin is relatively soluble in the cold unless it is complexed with fibrin and fibrinogen^{7,23–25,27,28}.

Antisera raised against the plasma or cell surface forms of fibronectin readily cross-react, and antibodies specific for only one form have not yet been described^{13,27,29–31}. Monospecific anti-fibronectin antisera against either form specifically immunoprecipitate only fibronectin from cell homogenates^{32–34}. Current information summarised below suggests that although the two forms of fibronectin are very similar, they are probably not identical. Whether they are products of different genes, or of one gene and are modified post-transcriptionally, is not yet known.

Quantities and location

Cell surface fibronectin constitutes 1–3% of total cellular protein of embryonic or adult fibroblasts cultured *in vitro*³⁵. A few

permanent cell lines also display large amounts of fibronectin, but many other permanent fibroblastic lines have much smaller quantities that are detectable only by cell-surface labelling techniques³⁵. Cell surface fibronectin has also been found on astroglial cells³⁰ and on certain cultured epithelial cells^{36,37}, including blood vessel endothelial cells^{38,39,114}.

Cell surface fibronectin is located in striking fibrillar arrays on the surface of many cultured cells, as determined by immunolocalisation studies with anti-fibronectin antibodies (Fig. 1)^{3,6,29,30,32–46}. Cell surface fibronectin extends between cells, and is also found between cells and the tissue culture substratum^{3,29,32–34}. This localisation is consistent with a role in cell–cell and cell–substratum adhesiveness. In densely confluent cultures, cellular fibronectin forms a complex, interconnected extracellular matrix that may contain collagen or elastin^{3,10,12,29,32–46}. Electron microscopic localisation studies confirm that most of the fibronectin is present in extracellular fibrils and aggregates, with only small amounts present diffusely distributed along the outer surface of the plasma membrane of cells^{37,43–45,47}. Most cellular fibronectin can be detached and separated from plasma membranes centrifuged in sucrose gradients^{48,49}, and as much as half can be released from living cells incubated in 1M urea^{50,51}. Most of the protein therefore seems to be loosely associated with the membrane. In addition,

Fig. 1 Cell surface fibronectin on chick fibroblasts *in vitro*. A confluent culture was fixed and stained with affinity purified anti-fibronectin followed by fluorescein-labelled anti-immunoglobulin. $\times 520$.



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large amounts of cellular fibronectin are also continuously secreted or sloughed from cells into culture media^{10,20,30,37,53-58}

Fluorescently labelled antibodies have also been used to determine the locations of the fibronectins in embryonic and adult tissues. Fibronectin is present as extracellular fibrils in connective tissue, in primitive mesenchyme, and in many basement membranes^{59,60}. During embryonic development, fibronectin is first detectable on the cells of the blastula inner cell mass⁶¹. Later in development, fibronectin is lost as mesenchymal cells differentiate into muscle, cartilage, and renal tubular epithelium^{59,62}. It is not present in hepatocytes^{59,60}, in contrast to many other plasma proteins. Fibronectin is also present in amniotic and cerebrospinal fluids^{37,63,64}, and on platelets²³. Since anti-cell surface and anti-plasma fibronectins cross-react, it is important to note that most of these immunological studies do not indicate whether the fibronectins at each of these sites are the cellular or plasma form.

Plasma fibronectin (cold-insoluble globulin) is present in human plasma at 0.3 mg ml⁻¹ (ref. 23). As it binds to fibrin, its concentrations are lower in serum²³. Plasma fibronectin also binds to heparin, and together with fibrinogen will also form a cryoprecipitate⁶⁵. Plasma fibronectin can be covalently cross-linked to fibrinogen or to itself by plasma transglutaminase (activated factor XIII)^{25,26}. Factor XIII can also covalently crosslink cellular fibronectin to itself^{66,67}.

Concentrations of fibronectin in blood are decreased after extensive trauma, in the disseminated intravascular coagulation syndrome, and after reticuloendothelial system blockade following injection of colloids in animals⁶⁸⁻⁷². Levels of the protein are often elevated in cirrhosis and the cholestasis of pregnancy, possibly because of defective hepatic removal⁷³.

Decrease after malignant transformation

A major source of interest in fibronectin is the finding that the amount of cell surface fibronectin is usually decreased after cell lines are transformed by oncogenic viruses or carcinogens, and on cultured tumour cells (Table 3). However, there are exceptions to this generalisation, particularly among spontaneously transformed cell lines (Table 3).

The tumorigenicity (capacity to form benign or malignant tumours after injection into immunosuppressed or immune-compatible animals) of cell lines generally correlates with decreases in cell surface fibronectin^{29,45,99,100}, although a recent study of closely related transformed cells grown in semi-solid medium has cast doubt on this relationship (P. H. Gallimore, J. K. McDougall, and L. B. Chen, personal communication; see also ref. 95). Instead, decreases in this protein may correlate more with the propensity of cells to metastasise⁴⁵.

The decreases in cell-associated fibronectin after malignant transformation are 5- to 7-fold in chick fibroblasts and more than 10-fold in human fibroblasts and glial cells^{30,52,55}. However,

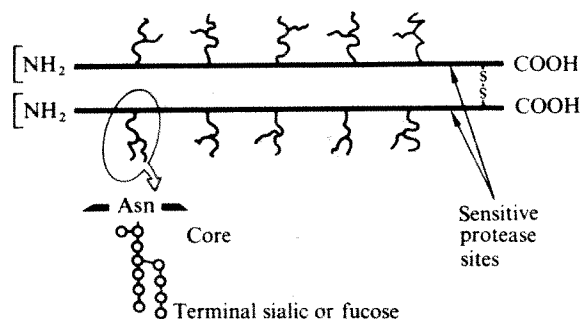


Fig. 2 Current model of fibronectin structure. Note elongated shape and distribution of inter-subunit disulphide bonds, adjacent sites that are highly sensitive to the proteases plasmin and trypsin, and the blocked amino termini. The oligosaccharides consist of a core of N-acetylglucosamine and mannose linked to asparagine residues with distal galactose residues that may be linked to terminal sialic acid or fucose residues.

Table 1 Nomenclature of glycoprotein factors

Cold insoluble globulin ⁷
Anti-gelatin factor ⁸
Microfibrillar protein ^{9,10*}
Opsonic protein ¹¹
Fibroblast surface antigen (SFA) ^{12,13*}
Galactoprotein a ^{14,15*}
Cell attachment factor (CAF, c-CAP) ¹⁶⁻¹⁸
Large, external, transformation-sensitive (LETS) protein ^{19*}
Cell surface protein (CSP) ^{20*}
Zeta (Z) ^{21*}
Cell spreading factor ²²
Fibronectin ¹

Asterisks indicate cellular fibronectin.

decreases of more than 10-fold in cell surface fibronectin also accompany the establishment of several permanent rodent cell lines and these decreases are not accompanied by the acquisition of tumorigenicity³⁵. The potent tumour promoter phorbol myristate acetate also causes a reversible loss of fibronectin¹⁰¹.

It is obviously important to resolve whether there is any direct causal relationship between decreases below specific quantities of cell surface fibronectin and the propensity of cells to form tumours or to metastasise.

Functions of the fibronectins in adhesion

Fibronectins are thought to be involved in a wide variety of cellular activities (Table 2). The simplest unifying interpretation of these findings is that the fibronectins function as adhesive proteins that bind cells to other cells or to substrata (Fig. 3). This conclusion is based on experiments *in vitro* utilising model systems of cellular adhesion, in which purified fibronectin is found to increase the adhesion of cells to cells or to the substrata on which they are grown.

The first evidence suggesting that cellular fibronectin may play a part in cell-cell adhesiveness was the finding that fibronectin purified from cell surfaces behaves like a lectin and readily agglutinates fixed sheep erythrocytes in concentrations as low as 1 µg protein per ml (refs 50, 58). Purified cellular fibronectin binds directly to fixed erythrocytes and can be recovered intact⁵⁰. Proteases and chelating agents destroy the agglutinin activity; these agents are known to disrupt cell-cell adhesion⁵⁰. Purified cell surface fibronectin can also increase the aggregation of living dissociated chick embryo and baby hamster kidney (BHK) cells¹⁰².

Purified cell surface fibronectin can also mediate the attachment of dissociated cells to collagen^{17,102}. Treatment with cellular fibronectin also increases the extent or attachment or the resistance to detachment of several transformed cell lines to plastic tissue culture substrata^{56,103,104} (see also ref. 105).

Serum factors are required for the attachment of certain cells to collagen-coated dishes^{16,17,106,107}, or for cells to attach and spread on tissue culture dishes¹⁰⁸⁻¹¹¹. These cell attachment and cell spreading factors are now thought to be serum fibronectin (cold insoluble globulin)¹¹¹⁻¹¹³. Such serum factors can bind directly to collagen or tissue culture dishes in the absence of cells^{16,17,106,108-110}. The fibronectin-mediated attachment of cells to collagen or spreading on to plastic substrata requires divalent cations such as calcium and the expenditure of cellular metabolic energy^{16-18,106,108}. Certain cells with high levels of cell surface fibronectin do not require serum fibronectin for spreading⁵. These cells may use cell surface fibronectin, since cellular fibronectin is equally active in promoting cell spreading and attachment to collagen¹⁷².

Both plasma and cell surface fibronectins can be purified by affinity chromatography on columns to which denatured collagen has been covalently coupled^{102,107,115-117,123}. Denatured collagens types I, II, and III are substantially more effective in binding plasma fibronectin than native collagens^{116,117}. The specific sites of interaction of plasma fibronectin with denatured collagen consist of a strong binding site at residues 643-819 of

the collagen $\alpha 1$ chain, plus several other weaker sites^{117,118}. Type III is the most effective native collagen in binding plasma fibronectin^{116,117}. The physiological significance of these differences in binding is not yet known.

Plasma fibronectin may play an important part in the removal of collagenous and other debris from blood after injury via the sessile macrophages of the reticuloendothelial system. An extensive series of experiments had identified an 'opsonic protein' in plasma that was involved in reticuloendothelial system clearance of injected gelatin-coated colloidal particles from blood^{11,69-71,119-121}. Other independent studies had identified an 'anti-gelatin factor' in serum that would bind to gelatin, and that was required for macrophage ingestion of denatured collagen^{8,122,123}. Both the opsonic protein and anti-gelatin factor have been purified recently, and both seem to be identical to plasma fibronectin (cold-insoluble globulin)¹²⁴⁻¹²⁶.

Are all of these biological activities of the fibronectins shared by plasma and cell surface forms? Recent experiments suggest that although both proteins are equally active in promoting cell spreading¹¹³ and attachment to collagen, cellular fibronectin is more effective in haemagglutination and in restoring adhesiveness and normal morphology to transformed fibroblasts (see below). Nevertheless, it is clear that both fibronectins display adhesivity.

Reconstitution and inactivation experiments

A potentially more general approach to determining the functions of the cell surface form of fibronectin is to reconstitute it experimentally on the surface of cells missing this protein, that is, on to transformed cells. These experiments can help to identify which of the alterations in cell morphology or behaviour occurring after cells are transformed could be due to the loss of this protein.

In 14 transformed fibroblastic cell lines examined to date, purified cellular fibronectin partially restores a more normal fibroblastic appearance to the cells^{44,56,102,103,127}. The effects include restoration of the flattened cell shape, elongated processes, smooth cell surface, and prominent intracellular bundles of microfilaments containing actin and myosin that are characteristic of normal fibroblasts *in vitro*^{44,56,102-104,127}. In these experiments, amounts of cell surface fibronectin equal to those present on freshly explanted fibroblasts can be restored to the transformed cells¹⁰³, although the reconstituted fibronectin may not bind as efficiently as originally¹⁰⁷. The reconstituted fibronectin seems to be present in normal sites as evaluated by immunofluorescence^{34,56} and by its capacity to undergo normal antibody-induced redistribution after incubation with u-fibronectin antibodies³⁴ (see below).

Table 2 Biological activities of the fibronectins

Cell-cell aggregation
Fixed erythrocytes
Live cells
Cell-substratum adhesiveness
Collagen substratum*
Plastic substratum
Cell spreading*
Reversion of transformed phenotype
Cell alignment and overlapping
Fibroblastic morphology
Cell surface morphology
Microfilament bundle organisation
Increased cell motility
Reticuloendothelial system clearance of colloids
Liver
Isolated macrophages
Specific binding to macromolecules
Collagen (denatured and type III native)*
Fibrin and fibrinogen
Heparin

Asterisks indicate activity known to be shared by both plasma and cellular fibronectins.

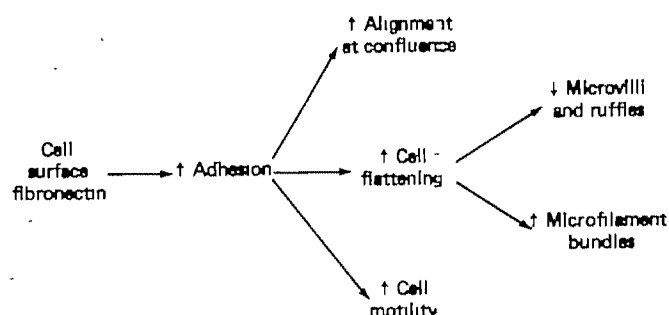


Fig. 3 Suggested relationship of cellular fibronectin to several biological activities (see refs 3, 102, 171 for discussion of evidence for this hypothetical scheme). The central activity of fibronectin would be to increase cell-cell and cell-substratum adhesion.

Besides affecting morphology, fibronectin treatment can restore a more normal social behaviour *in vitro*. For example, transformed fibroblastic cells tend to grow in relatively random patterns with cell bodies criss-crossing over one another, whereas normal fibroblasts tend to align in parallel arrays of cells. Fibronectin-reconstituted transformed cells show a restoration of this social alignment in approximately half of the cell lines tested^{56,102,103}. This defect in social behaviour is a common characteristic of cells transformed by oncogenic viruses and may result from a decrease in cell adhesion or to changes in specific cell recognition events such as contact inhibition of movement¹²⁸⁻¹³⁰. Another factor involved in maintaining cell alignment is released from cells by 0.2 M urea treatment¹³¹; however, this factor and fibronectin are clearly different molecules^{20,173}.

Fibronectin-treated cells also migrate more rapidly, both as single cells or as masses of cells migrating out from cell aggregates^{102,132,133}. These effects could be the result of establishing more effective cell-to-substratum traction¹³², or of a more asymmetric cell shape that promotes directed cell movements^{35,56,103,133}.

A complementary approach to investigating the biological activity of fibronectin is to incapacitate it with proteases or anti-fibronectin antibodies. Treatment of chick fibroblasts with purified antibody results in a rapid alteration in cell shape to a rounded morphology resembling that of many transformed cells that are deficient in cellular fibronectin³⁴. Cells treated with proteases that do not remove fibronectin tend to aggregate, whereas its removal results in cell retraction without such aggregation¹³⁴. These results are also consistent with a role for fibronectin in cell adhesion.

Cellular fibronectin does not appear to directly control cell growth rates, nutrient transport, or cyclic AMP levels. For example, these changes could not be reverted to normal after fibronectin treatment of transformed cells^{44,56,103,135}.

Protease experiments also provide additional evidence against a direct role for fibronectin in growth regulation. For example, treatment with papain or thermolysin removes all detectable fibronectin from the cell surface, but does not activate growth of chick fibroblasts¹³⁴. Conversely, thrombin activates cell division without removing cellular fibronectin^{136,137}. Insulin treatment can also stimulate mitosis without decreasing fibronectin⁷⁸.

Structure and composition

A current model for the structure of the fibronectins is shown in Fig. 2. Both plasma and cell surface fibronectins are probably elongated rather than globular proteins^{24,26,138} (contrast ref. 6). Plasma fibronectin is a dimer of two polypeptides of 220,000 each^{24,26}. Its subunits may not be identical, since analysis of plasma fibronectin after reducing disulphide bonds reveals a closely spaced doublet band^{24,26}. Cell surface fibronectin exists both as disulphide-bonded dimers and multimers^{31,139,140}. The interchain disulphide bridge(s) of both forms of fibronectin are confined to one end of the molecule, probably the carboxyl

Table 3 Effects of transformation on cell surface fibronectin

Species and cell type	Agent		Refs
	Fibronectin decreased	Not decreased	
Chicken			
Embryo fibroblast	Rous sarcoma virus (8), B77 avian sarcoma virus, avian erythroblastosis virus	—	15, 40, 49, 53, 55, 74–80
Hamster			
Embryo fibroblast	Carcinogen (3)	—	81
Embryo kidney	Adenovirus	—	29
Nil	Hamster sarcoma virus, polyoma virus	—	8, 82–84
	Spontaneous (9)	Spontaneous	18
BHK	Polyoma	—	82, 85, 86
Tumour explant	Spontaneous	—	29
Human			
Embryo fibroblast	SV40	—	52
Tumour explants	Spontaneous (13)	—	29, 30, 52, 87
Mouse			
Embryo cells	—	Spontaneous	88
3T3	SV40, polyoma virus, Moloney sarcoma virus	SV40 cell mutant, polyoma virus	89–91
C3H/10T 1/2	Carcinogen	Carcinogen (3)	92
Explants	Spontaneous (3), [L929]	Spontaneous (2), [L929]	29, 87, 93–95
Tumour explants	Spontaneous (11), carcinogen	—	29, 87
Rat			
Embryo fibroblast	Adenovirus (4), Rous sarcoma virus	Adenovirus	29
F111 fibroblast	Carcinogen (2)	Polyoma virus	96
NRK	Rous sarcoma virus, B77	—	74, 97
Embryo myoblast	Adenovirus	—	29
L8 myoblast	Rous sarcoma virus (3), B77	—	98
Tumour explants	Spontaneous (2)	—	29
Totals	77	12	

Numbers in parentheses indicate the number of different strains or independent variants of each agent examined, if greater than 1.

terminus^{26,102,107,141,142}. This asymmetric location may serve to increase the asymmetry of the molecule. Reduction of disulphide bonds inhibits the effects of cellular fibronectin on cell–substratum adhesion¹⁴³. The amino termini of both forms are blocked^{24,26,51}, and their amino acid and carbohydrate compositions are very similar^{24,51,144,145}.

Information about the secondary and tertiary structures of these proteins are provided by circular dichroism and fluorescence measurements, respectively. Circular dichroism reveals little evidence for regular secondary structure in both molecules, since the optical activity is unusually low^{72,138}. Nevertheless, some folded regions appear to be present, since the tryptophan fluorescence spectrum suggests that at least some of the tryptophan residues are buried in hydrophobic regions of structure¹³⁸. Heating exposes the tryptophan sidechains, and destroys fibronectin's agglutinating activity^{50,138}. The fibronectins therefore appear to be floppy, asymmetric molecules, with localised regions of folding that may be important for adhesive activity.

Direct comparisons of the structures of fibronectins isolated from plasma and culture medium (which contains cell surface fibronectin secreted by cells) reveal very similar isoelectric points, migration in SDS–polyacrylamide gels, and cyanogen bromide cleavage products for proteins from these sources¹⁴⁵. However, there are several reports of slight differences in electrophoretic mobility between cellular and plasma fibronectins^{37,67,139}, and in some gel systems, the plasma form can be resolved as a doublet, whereas the cellular form is a single broad band^{20,24,26,37,172}.

The solubilities of the two proteins also differ. The plasma form is soluble at neutral pH²⁴, whereas the cellular form is insoluble unless the pH is elevated to pH 11 or it is treated with strong denaturing agents^{13,51,146,147}. The insolubility of cellular fibronectin may account for its aggregated distribution on the cell surface.

What accounts for these differences in solubility and electrophoretic mobility? Differences may exist in the amino termini of these proteins. For example, the differences in electrophoretic mobilities between these proteins are preserved even after the putative carboxyl terminal disulphide bonds are cleaved from the molecule by limited proteolysis with trypsin¹⁷². Human and bovine plasma fibronectins have amino terminal pyroglutamate residues^{24,26}, whereas chick cellular fibronectin does not⁵¹. One current speculation is that the two forms of fibronectin may be the same gene product, but the surface form is proteolytically processed to the slightly smaller plasma form. Alternatively, they may be produced by separate genes arising from gene duplication. Several laboratories are actively investigating these possibilities.

Carbohydrates of the fibronectins

The carbohydrates on cell surface fibronectin consist primarily of the 'complex' type of oligosaccharide linked by *N*-glycosidic bonds to asparagine. There are probably an average of 4–6 such oligosaccharide groups per molecule, containing terminal galactose, sialic acid, and fucose residues^{51,148,149}. The structure of the carbohydrate moieties of the plasma form of fibronectin are not known, although the compositions of the two forms are similar^{24,51,145,149}.

Treatment of cells with the antibiotic tunicamycin specifically blocks the formation of this class of oligosaccharide. Chick fibroblasts treated with tunicamycin produce unglycosylated fibronectin, and total amounts of this protein are substantially decreased^{148,150}. The decrease is not due to altered synthesis or secretion, but instead to increased proteolytic degradation¹⁴⁸. These results suggest that for cellular fibronectin, carbohydrates are not necessary for synthesis, processing, or secretion, but instead may function to protect the protein from proteolytic attack by cellular proteases.

Regulation of fibronectin

Quantities of cell surface fibronectin vary as cells traverse the cell cycle. Fibronectin levels are lowest on cells in mitosis by both radioactive surface labelling and immunofluorescence criteria, even though intracellular levels of fibronectin do not appear to vary significantly^{19,43,86,93}. This finding is of interest, since cells in mitosis also resemble transformed cells with respect to morphology, agglutinability by lectins, glycopeptide size, and loss of contact inhibition of movement¹⁵¹.

Even though fibronectin is decreased on cells during mitosis, the turnover rate of cell surface fibronectin is substantially longer than the cell cycle in chick cells (half remains after 36 h with an 18 h generation time; refs 55, 152; see also refs 21, 77, 86, 153). This apparent paradox could be explained if cells temporarily release themselves from the matrix of cell surface fibronectin during mitosis, and spread back into and rebind the same matrix as they re-flatten following cell division.

Growth conditions also influence quantities of cell surface fibronectin. For example, nonproliferating cells in dense cultures in the G1 phase of the cell cycle generally have larger amounts of fibronectin than rapidly growing cells^{15,19,47,83,85,86,93,98}. Cells prevented from dividing by serum deprivation can have either elevated¹⁹ or substantially reduced amounts of cell surface fibronectin¹⁵⁴. Mouse 3T3 cells cultured in media containing lowered concentrations of serum have little fibronectin; cell surface fibronectin levels are restored after treatment with epidermal growth factor, but not by other hormones¹⁵⁴. Fibronectin production by human fibroblasts is also reportedly stimulated by thrombin¹⁵⁵.

How fibronectin becomes depleted on the cell surface after malignant transformation has been studied in detail. Increased cell proteases after transformation cannot alone account for the loss of fibronectin^{84,87,156}. In chick fibroblasts transformed by several strains of tumour virus, the major factor in the decrease was found to be decreased biosynthesis⁵⁵, apparently due to decreased amounts of translatable messenger RNA¹⁵⁷. However, other factors also contribute to the decreases in fibronectin, including an increased rate of proteolytic degradation^{21,55,77} and a decreased capacity to bind the protein to the cell surface⁵⁵. In hamster cells, major factors in the loss of fibronectin after transformation also appear to be reduced biosynthesis and increased degradation^{107,158}. However, in human transformants, decreased capacity to retain the protein on the cell surface may be more important than its decreased synthesis^{30,52}.

Amounts of fibronectin on the cell surface are also regulated during embryonic differentiation. As *in vivo*⁵⁹, differentiation of muscle or cartilage cells *in vitro* is accompanied by a decrease in fibronectin on the cell surface^{47,159,160} (contrast ref. 98). Dedifferentiation of chondrocytes results in increased amounts of fibronectin^{160,174}. Experimental addition of cellular fibronectin to chondrocytes results in cells morphologically similar to undifferentiated mesenchymal cells that no longer synthesise the large amounts of sulfated proteoglycans characteristic of differentiated chondrocytes¹⁷⁵. Programmed losses of cellular fibronectin may, therefore, be a prerequisite for differentiation of certain cells.

These studies on the regulation of fibronectin levels on the cell surface indicate that control can be exerted by a variety of events including altered growth conditions, transformation, hormonal stimulation, and embryonic differentiation. This regulation may occur through altered synthesis, turnover, or binding to the cell surface.

Relationship of fibronectin to cytoskeletal and membrane components

The overall organisation of cell surface fibronectin into fibrillar patterns parallel to the long axis of cells is roughly similar to that of intracellular microfilament bundles⁴⁰. However, immunofluorescence studies comparing fibronectin and actin localisation patterns have shown that the two patterns

need not coincide (ref 32 and our unpublished results). These findings suggest that there may not be direct transmembrane connections between microfilament bundles and all of the surface fibronectin.

However, indirect associations or discrete attachment points may occur. For example, cell surface fibronectin is concentrated on the lower surface of cells¹⁶¹, and particularly in the adhesive attachment sites of cells to substrata¹⁶². Treatment of cells with low concentrations of proteases or anti-fibronectin can result in cell rounding and in a loss of microfilament bundle organisation^{34,163}. Treatment of transformed cells missing microfilament bundles with fibronectin permits reorganisation of intracellular microfilament bundles^{56,104}, perhaps due to increased cell-substratum adhesion that permits anchoring of these bundles (Fig. 3).

Conversely, cytoskeletal elements may also affect the organisation of cellular fibronectin. Treatment of cultured cells with cytochalasin results in a release of fibronectin from the cells, suggesting a possible requirement for microfilament integrity in maintaining fibronectin organisation^{164,165}. Treatment of cells with anti-fibronectin can result in antibody-induced redistribution of fibronectin¹⁶⁶ to 'caps' of antigen on the upper surfaces of cells³⁴. This process requires metabolic energy, and is inhibited by a combination of colchicine and cytochalasin³⁴; cytoskeletal elements may also be involved in this redistribution. More detailed analyses of the ultrastructural and biochemical relationships of these proteins should provide further insight into fibronectin-cytoskeletal interactions.

Fibrillar networks of cell surface fibronectin are relatively immobile by comparison with mobility of other cell surface components. For example, receptors for concanavalin A move with a diffusion constant of $4 \times 10^{-11} \text{ cm}^2 \text{ s}^{-1}$, while fibronectin's is $< 5 \times 10^{-12}$ (ref. 42). As cellular fibronectin is relatively immobile, it might inhibit the mobility of other components of the cell surface. The movement of gold particles on the upper surfaces of 3T3 cells is inhibited by fibrils of cellular fibronectin¹⁶⁷. In contrast, the mobilities of a ganglioside analog, a lipid probe, and a mixture of surface antigens are not affected by the presence of fibronectin fibrils⁴². It is therefore possible that fibrillar networks of fibronectin affect interactions of cells with large objects such as particles, cell processes, or substrata, whereas smaller membrane components remain unaffected. These results are consistent with the location of fibronectin fibrils close to, but not necessarily tightly apposed to, the plasma membrane.

Future prospects

Although we know the overall structure, location, and probable general functions of the fibronectins, many important questions remain. For example, although the fibronectins are adhesive proteins, it is not clear how they function *in vivo*—for example in organising the extracellular matrix, cell migration, wound healing, macrophage ingestion of colloids, hemostasis, and embryogenesis. Is this protein directly involved in inhibiting tumorigenicity or metastasis *in vivo*? Is its role in the reticulo-endothelial system confined to the removal of collagenous debris, or does it play a wider role in host defense^{168,169}? Other interesting problems concern the precise differences in structure and regulation of the plasma and cell surface forms of fibronectin, their possible relationships to other more specific adhesive molecules that appear at precise times in vertebrate embryonic development¹⁷⁰, and the biophysical mechanisms by which these proteins can bind to cells and to substrata.

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articles

The significance of magnetospheric research for progress in astrophysics

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Recent in situ measurements have led to a substantial revision of our picture of the magnetosphere and parts of the heliosphere. This concerns the character and distribution of electric fields and currents, the ways in which charged particles are energised, and the chemical composition of the magnetospheric plasma. This revision reflects the fact that real cosmic plasmas behave in fundamentally different ways than predicted by the traditional, idealised models used in magnetospheric physics and in astrophysics. The new understanding of the general properties of cosmic plasma gives us an improved basis on which to interpret astrophysical observations.

ASTRONOMY, and especially astrophysics, is in an interesting phase, where remarkable observational discoveries are being made. However, since astrophysical phenomena occur in remote regions, where *in situ* observations are not possible, and involve matter in various extreme states, it can be difficult to understand the physical phenomena behind them. Usually the states involved are magnetised plasmas at extremes of temperatures and/or density. The interpretation of the observations then depends on whatever knowledge of plasma processes is available.

The difficulties involved can be understood by recalling those faced by thermonuclear fusion research 20 years ago. The task was to predict the behaviour of a very hot, magnetically confined, plasma. A well developed theoretical formalism existed. It was the classical theory of gases, generalised to include charged particles, but it had not been subjected to a thorough experimental verification. It was found that the hot plasmas in thermonuclear machines often behaved quite differently from what had been predicted from existing plasma theories. This necessitated a fresh start, with experiments as the backbone of the theoretical structures. However, even now the complicated behaviour of hot, magnetically confined plasma is far from being completely understood.

In astrophysics the situation is more difficult because, in contrast to the plasmas in thermonuclear machines, *in situ* 'diagnosis' of the astrophysical objects is not possible. The coming of the space age means that cosmic plasma can now be studied *in situ*, but only in our near-space environment.

To understand the significance of this, it is important to realise that our immediate environment in space, the magnetosphere (including the ionosphere) of the Earth and the adjacent interplanetary space, offer a rich sample of plasma

states in natural conditions. They include cool, weakly ionised, collision-dominated, gravity confined plasmas as well as hot, essentially fully-ionised and virtually collisionless magnetically confined plasmas. While the parameters may still be many magnitudes less than the extreme values attained in some remote astrophysical plasmas, the plasmas in the magnetosphere are a large step from the parameter range available in Earth-based laboratories. Study of the magnetospheric plasmas can, therefore, greatly widen the empirical basis of cosmic physics.

Spacecraft observations in the magnetosphere have already identified various plasma processes which were not predicted from classical kinetic theory, the MHD formalism or even modern plasma theories. In particular, as we point out below, limitations have been found to the validity of the classical concept of the frozen-in-magnetic field, which used to be a 'golden rule' in cosmic electrodynamics.

The need for an improved empirical basis in cosmic physics is dramatically illustrated by the history of magnetospheric research itself. Before *in situ* measurements were possible, and even for many years after satellite measurements had begun, the magnetosphere was described in terms of highly idealised models that were a heritage from classical plasma physics. They involved infinite conductivity along magnetic field lines, frozen-in-magnetic fields and simple magneto-hydrodynamic convection. It was tacitly assumed that the matter in the outer magnetosphere was (mainly) hydrogen ions from the solar wind, and that electric fields—if they were considered at all—were everywhere transverse to the magnetic field.

For a long time even spacecraft observations failed to reveal the fallacy of such assumptions, this was probably because their indiscriminant use guided the planning and design of the observations. Thus, for example, particle detectors in low Earth orbit always looked upwards because accelerated particles coming from below—as have been recently observed—were inconceivable in the prevailing theories.

Only during the past few years, has the magnetospheric physicist found that the classical concepts break down in certain interesting regions of the magnetosphere. Also, only during the past few years, have the significance of Birkeland currents (magnetic-field-aligned electric currents in the magnetosphere) and magnetic-field-aligned electric fields been realised. This has far-reaching consequences in understanding the aurora and other phenomena in the magnetosphere.

Although the understanding of the magnetosphere has increased gradually a veritable breakthrough has resulted from a few particle and field measurements on one single satellite,

S3-3. We now know that: (1) the frozen-field condition is not reliable, and different levels of the magnetosphere can be electrically decoupled—at least partially; (2) magnetic-field-aligned electric fields with potentials of several kV exist not only in narrow regions but over hundreds of km in latitude; (3) ionospheric ions are accelerated along the geomagnetic field into the magnetosphere; and (4) large, sometimes dominant part of the storm time ring current consists of oxygen from the ionosphere rather than hydrogen from the solar wind.

Such discoveries in magnetospheric physics indicate that the really important phase of magnetospheric research, in terms of fundamental physical understanding of plasma behaviour in natural conditions, has only just begun.

This will also have far-reaching consequences for the foundations of cosmic plasma physics and hence for astrophysics. If a cosmic plasma system which is so close to us could, for such a long time, be misinterpreted in terms of the physical processes going on, how confident can we be of the interpretations of the astrophysical phenomena known only by remote observations? Kelvin estimated the age of the Earth, on the basis of its cooling rate, to be 50 Myr because radioactivity was not known during his generation. Inadequate plasma physical models could lead to—and have already led to—similarly misleading conclusions in astrophysics.

It is worth emphasising the importance of laboratory experiments in cosmic plasma physics. In thermonuclear research a great deal of progress has been possible, after the initial setback, because a large experimental effort has guided the theoretical development. Similarly, in cosmic plasma physics, laboratory experiments, as well as magnetospheric research, are needed to guide the theoretical interpretation of remote observations. One example is the electric double layer phenomenon, which according to recent observations is likely to occur in the magnetosphere and may have profound consequences in cosmic plasmas. Although its existence has long been known in unmagnetised plasma, and recently proved in magnetised plasma, an adequate theory—especially of its dynamics—does not yet exist, and in all applications one must rely on results of laboratory experiments.

The role of magnetisation

Virtually any known astrophysical plasma is magnetised. To be precise, a magnetised plasma is defined as a plasma that contains a magnetic field which is strong enough to make the gyroradii of all its charged particles much smaller than the linear dimensions of interest. As the latter are usually large in cosmic plasmas, even a small magnetic field is sufficient to make the plasma magnetised.

For example, for stellar dimensions and temperatures of the order of 10^6 K, as in the corona, magnetic fields above 10^{-12} T are sufficient. For galactic dimensions, and even if we consider the highest observed energies, the corresponding number is 10^{-17} T. The smallest cosmic magnetic fields which can be measured or inferred are of the order of 10^{-9} T.

For magnetised plasmas it is often useful to distinguish between three classes: high, medium and low density plasmas¹. They are defined in terms of the relative magnitudes of the gyroradius, ρ , the mean free path, λ , and the characteristic linear scale, l_0 , as follows:

High density, $\lambda \ll \rho$; medium density, $\rho \ll \lambda \ll l_0$; low density, $l_0 \ll \lambda$.

High density plasmas occur in stellar bodies, lower stellar atmospheres and lower planetary ionospheres. Except for wave propagation they have a high degree of isotropy. Current densities and electric fields obey a simple local relationship—Ohm's law.

Medium density plasmas prevail in fairly thin transition regions above the lower stellar atmospheres and planetary ionospheres, but can also occur in regions such as the Earth's plasmasphere. These plasmas are anisotropic in terms of

diffusion and electric current conduction. The electric field-current density relation is still local and can be expressed as a generalised Ohm's law.

Low density plasmas prevail throughout most of the Earth's (and other) magnetosphere(s), the solar corona, interplanetary, interstellar and intergalactic space. They are the most complicated plasmas, because collective interactions tend to be important instead of the relatively simple binary collisions that prevail in low and medium density plasmas. Numerous kinds of plasma instabilities and wave-particle interactions are possible. In terms of electric properties, a major complication is that there need no longer exist any local relationship between electric field and current density. The reason is that charge carriers may gain momentum from the electric field in one region and lose it, for example, by magnetic mirroring, in a different region. Low density plasmas in this sense are a major preoccupation of modern plasma physics, especially in thermonuclear research where $\lambda \gg l_0$ is one of the requirements for magnetic confinement.

In the magnetosphere, the most exciting discoveries in recent years are related to the properties of low density plasmas. The consequences for the understanding of cosmic physics in general can be expected to be far reaching.

Electric currents and fields in cosmic plasmas

The magnetisation of all cosmic plasmas is connected with electric currents through Maxwell's first equation $\text{curl } \mathbf{B} = \mu_0 \mathbf{j}$ and as cosmic magnetic fields are not generally curl-free, a fundamental problem is how electric currents are carried by cosmic plasmas².

For high and medium density plasmas this is a simple problem, because the current is determined essentially by a local balance between electric, magnetic and frictional forces. The current-carrying ability of the medium at each point is characterised by a conductivity which in high density plasmas is a scalar, in medium density plasmas a tensor.

In low density plasmas the conduction of current is complicated. This fact is often overlooked, and classical conductivity formulas are erroneously applied to low density cosmic plasmas. One illusion is that, as collisions become rare and vanish, the conductivity along magnetic field lines increases and becomes essentially infinite. In reality, the concept of conductivity will lose its meaning¹. There need not be any local relation such as Ohm's law.

Magnetospheric spacecraft observations in the past few years have shown that the conductivity concept is obviously misleading. This has great consequences in many astrophysical applications, too. In particular, the ability of a low density plasma to carry electric current is far from being 'infinite', and substantial electric fields can be required to drive the Birkeland currents (magnetic-field-aligned currents) of the magnitude (of the order of 10^{-6} A m⁻²) observed in the magnetosphere. This greatly affects astrophysics, because first it changes the whole electrodynamic of cosmic plasma; second, the finite electric field along magnetic field lines violates the concept of frozen-in magnetic field lines, and finally it provides an efficient means of particle acceleration.

A few typical phenomena will now be briefly described that involve substantial magnetic-field-aligned electric fields.

Anomalous resistivity has been recognised for over a decade as a possible means of supporting magnetic-field-aligned electric fields in space plasma (for a review see ref. 3). Anomalous resistivity is characterised by wave-turbulence sufficiently strong to impede the current-carrying electrons so that a local Ohm's law is reestablished. The wave turbulence is usually considered to be driven by the current itself, but may also be maintained by a separate agent such as a high-energy electron beam that is unstable in the local plasma environment^{4,5}. Whatever the cause may be, the d.c. electric field strength, E , that can be supported is limited by the condition $E \ll E_{\text{max}}$,

where E_{rms} is the r.m.s. value of the electric field of the wave turbulence⁷. This has been confirmed by direct measurements in the magnetosphere⁸.

The wave turbulence will act differently on particles in different parts of velocity space. Particles of sufficient initial velocity (in the direction of the electric force) will become runaways, that is, decoupled from the main population of charge carriers, and the current they carry will not be determined by local conditions but depend on the whole electric circuit involved. Therefore, if the runaway current becomes substantial compared to the total electric current, the local relationship between electric field and current is lost. Thus the current is not determined by local 'resistivity', and the whole circuit must be taken into account. Then the term 'resistivity' loses its meaning.

The anomalous resistivity phenomenon has been established by laboratory experiments (for a review, see ref. 9) and wave fields measured in the magnetosphere (for a review, see refs 10, 11) are large enough to support magnetic-field-aligned electric fields of the order of mV m^{-1} , which may integrate to voltage drops of the order of several keV, a field large enough to be of significance to magnetospheric plasma dynamics. On the other hand, in the magnetosphere, anomalous resistivity may not be the only, or even the most important, mechanism for supporting magnetic-field-aligned electric fields. Alternative mechanisms are the double layer and the magnetic mirror effect.

The electric double layer was discovered by Langmuir in 1929 but wide interest in the phenomenon has been revived recently by experiment studies¹²⁻¹⁴. For cosmical applications it is essential that its existence in magnetised plasma has now been proved^{15,16}. (For a review with special reference to possible space applications, see refs 17, 18.)

Like anomalous resistivity, a double layer is typically established as a consequence of current-driven plasma instabilities. To predict whether the end result of a given instability will be anomalous resistivity or a double layer is difficult. Theoretical studies have therefore often started with the assumption that the end state is one of anomalous resistivity.

The laboratory experiments which show that the result of the instability may well be a double layer instead of anomalous resistivity, illustrate the danger of a purely theoretical approach and the need for empirical experiments on the behaviour of the real plasma.

The electric potential drop across a double layer depends on factors such as density and temperature of the adjoining plasmas and on the electric properties of the circuit in which the current is closed. Thus, unlike anomalous resistivity, the electric double layer does not imply a local relationship between electric field and current density.

Double layers and anomalous resistivity differ in the way that the dissipated energy is deposited. In the case of anomalous resistivity, it is deposited in the ambient plasma at the location of the voltage drop, leading to intense local heating there. In the case of a double layer, all particles passing through the layer are 'runaways', and form beams that carry away the dissipated energy to be deposited in some other region where the beam is finally stopped. In the magnetosphere, this may account for the energy deposition in the polar ionosphere by auroral primaries without excessive heating in the acceleration region. In many astrophysical applications such remote deposition of energy may be very important.

Direct observations of double layers are difficult because they are relatively thin, 10–100 Debyelengths. The probability of encountering a double layer is one in several hundred satellite passes. In the recent electric field data of the S3-3 satellite⁸ there is one case (from about 200) where the magnetic-field-aligned electric field was reported to be several hundred mV m^{-1} . This is compatible with an electric double layer¹⁹.

In a low density plasma situated in a magnetic mirror field slight differential anisotropies between positive and negative

particles can lead to large magnetic-field-aligned electric fields even without a net current¹. In the magnetosphere where large field-aligned currents (Birkeland currents) flow to and from the auroral oval, and in many astrophysical situations, the magnetic mirror effect puts an upper limit to the conductance per unit area—a conductivity need still not exist—which above the auroral region of the Earth is at most of the order of a few $\text{A m}^{-2} \text{keV}^{-1}$ (refs 7, 20). This requires that the loss cone of the source plasma is continually replenished. If it is not, the current will be choked and the conductance reduced to still smaller values and can in principle approach zero.

The frozen field condition

If the classical conductivity formula were applicable to cosmic plasmas, the condition for 'frozen-in magnetic fields' would almost always be fulfilled. But the plasma properties described above may well decouple the electric fields in different portions of a magnetic flux tube (magnetic field unfreezing). This occurs at least in a limited region of the magnetosphere above the auroral ovals. Recent observations of comet tails²¹ are apparently the first proof of magnetic-field unfreezing in plasmas outside the Earth's magnetosphere.

Magnetic field unfreezing may also invalidate the law of isorotation, and in the context of planet and satellite formation allow an important phenomenon called partial corotation²².

Particle acceleration

Cosmic plasmas can generate suprathermal and high energy particles. In ordinary particle accelerators, insulators and sometimes also tuned harmonic signals are requisites for accelerating charged particles to high energy. None of these requisites are available in cosmic plasmas. Yet we observe cosmic ray particles with energies of 10^{10} eV or more, solar protons of 10^{10} eV and magnetospheric protons of $1\text{--}10^6$ eV. It would be difficult to generate such high-energy particles if the plasma had the simple magnetohydrodynamic properties usually ascribed to it.

Some particle energisation processes in cosmic plasma can be studied in the magnetosphere. Their products range from slightly suprathermal electron fluxes in the ionosphere to ions of several MeV in the radiation belts and in the tail region of the magnetosphere. Some of these acceleration processes involve wave fields due to various instabilities. Others depend on slowly fluctuating transverse electric fields to cause drift-resonant adiabatic acceleration, which can be compounded with non-adiabatic effects. An interesting process is the direct acceleration of charged particles in the magnetic-field-aligned electric fields that regularly occur in low density plasmas. This possibility was previously excluded by the assumption that magnetic-field-aligned electric fields cannot exist and that the frozen-field condition is always valid. In the magnetosphere, such a direct acceleration process seems to play an important part in auroral phenomena^{7,17,18,23,24}. In the solar atmosphere the field-aligned acceleration may also play an important part in flare phenomena²⁵, which is widely recognised to have many similarities with magnetospheric substorms²⁶. Field-aligned acceleration may also be important in many other cosmic plasmas. One example on a large linear scale is double radio sources²⁷. The energy, originating in the unipolar dynamo of the central galaxy, is carried by electric current to the two opposite remote regions where the current becomes unstable and a parallel electric field is established (presumably in a double layer).

The structure of matter in space

One of the most important results of space exploration is the discovery of a number of boundary surfaces. Some of these layers are due to electric sheet currents which may reverse the magnetic field. This often occurs in the magnetopause, in the neutral sheets of the magnetotail, in the solar equatorial plane² (previously interpreted as 'the sector structure'), and in

cometary tails²¹. Such boundary sheets may be very thin, sometimes only a few Larmour radii. Their persistence demonstrates that they have a high degree of stability and that cosmic plasmas have a general tendency to divide themselves into compartments by setting up such layers.

The discovery of the many boundaries separating different plasmas is remarkable in that neither theoretical considerations nor laboratory experience had led us to expect layers with the observed properties (although other types of layers were predicted). This is very important for our picture of the structure of space. We find that space occupied by plasmas tends to be divided in 'compartments', often separated by thin current sheets. On the interstellar and intergalactic scale, space may have a cellular structure consisting of many separate regions, containing plasmas of different magnetisation, density, temperature, and perhaps also different kinds of matter (koino- and antimatter).

Conclusions

For a proper understanding of astrophysical phenomena, the theoretical work must be guided by empirical knowledge of how plasmas behave in nature. Laboratory experiments have an essential role in this context but *in situ* observations of the magnetospheric plasma can, in some respects, provide us with an even better knowledge of plasma behaviour in natural conditions. The plasma in the magnetosphere (including the ionosphere) and the solar wind are the only cosmic plasmas accessible to extensive *in situ* observations and experiments.

Observations of magnetospheric plasmas extend our empirical knowledge to a new range of plasma parameters by many powers of 10. It is also fortunate that the Earth's magnetosphere contains such a rich variety of plasmas, from the relatively dense and cool ionospheric plasma to the extremely thin and hot plasmas at larger distances.

The truly fundamental progress in the understanding of the

magnetosphere has only just begun. It has begun because of the recent observational discoveries which mark a new phase of magnetospheric research. The knowledge already obtained, and the insights still to be gained, in this new phase of magnetospheric research should be of great value in understanding astrophysical plasmas and may lead to similar drastic revisions in astrophysics.

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Young's double beam interference experiment with spinor and vector waves

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The fringe shift observed in Young's double beam interference experiment using unpolarised neutrons, when one beam traverses a magnetic field before recombination, has been interpreted as implying direct observation of the sign reversal of a spinor wave function subjected to a 2π rotation. This interpretation is unsatisfactory because for any fermion the relative rotation of the spins in the two beams and the interference pattern are mutually incompatible observables. This incompatibility does not arise in the case of bosons which possess a state of zero spin-component. Furthermore, equivalent 'sign-reversal' effects may be observed in interference phenomena associated with any two-state system and a realisable photon analogue is proposed.

RAUCH ET AL.¹ and Werner *et al.*² have independently described neutron analogues of Young's double slit optical interference experiment using unpolarised neutrons which have been interpreted as a direct observation of the sign rever-

sal of a fermion wave-function under a 2π rotation. In these experiments, amplitude division of a coherent neutron wave train is brought about by a symmetrical Laue reflection in a single crystal of silicon. One of the emergent beams traverses a uniform magnetic field while the other propagates in a field-free region. The crucial result is the observation that the interference pattern formed where the two beams recombine, shifts through half a fringe when the magnetic field is varied from zero to a value such that the spins of those neutrons moving through the magnetic field precess through an angle of 2π relative to the spins of those neutrons which move in the field free region^{3,4}.

Interference pattern

This extremely elegant technique provides an interferometric method of distinguishing fermion from boson⁵ because the equivalent boson interference pattern would shift through a full fringe. One must, nevertheless, be cautious about the claim² that "we have directly observed the sign reversal of the wave function of a fermion produced by its precession of 2π radians in a magnetic field using a neutron interferometer". The difficulty relates to the notion of 'relative rotation'³⁻⁷ as applied

to the fermion spins in the context of an interference experiment as, for fermions, simultaneous observations of the relative rotation of the spins in the two beams and of the phase shift are incompatible. We may, of course, measure the angle of rotation of those neutron spins constrained to move in the magnetic field relative to the spins of neutrons in the field-free region. An essential feature, however, of all interference phenomena is that, whereas the wave amplitudes propagate along distinguishable paths, all information concerning which path the neutron actually follows is lost⁶. In these circumstances the notion of relative rotation ceases to have a meaning as it corresponds to nothing which is measurable. The objection therefore is founded on the uncertainty relation and is not pedantry because, as we show below, no equivalent inconsistency arises in the case of bosons.

Let u_0 be the incident spinor, then the neutron wave train reaching the detector in the absence of the interferometer is represented by the spinor $u_0(x) = u_0 e^{i\phi_0 x/\hbar}$. When the interferometer is introduced $u_0(x)$ is transformed to

$$u(x) = \begin{bmatrix} A + B e^{-i\alpha/2} & 0 \\ 0 & A + B e^{i\alpha/2} \end{bmatrix} u_0(x) = M u_0(x) \quad (1)$$

where A and B are real parameters characterising the amplitudes of the interfering beams and $\beta = -geBx/2p_0$ (ref. 3). The action of the interferometer is described by the transfer matrix

$$M = \begin{bmatrix} T^{1/2} e^{-i\alpha/2} & 0 \\ 0 & T^{1/2} e^{i\alpha/2} \end{bmatrix} \quad (2)$$

where T , the matrix determinant, is given by

$$T = A^2 + B^2 + 2AB \cos(\beta/2) \quad (3)$$

and

$$\tan\left(\frac{\alpha}{2}\right) = \frac{B \tan(\beta/2)}{B + A \sec(\beta/2)} \quad (4)$$

As M describes a transformation to a state whose normalisation has been multiplied by $T^{1/2}$, we may interpret T as the transmission coefficient for the interferometer. It follows from equation (3) that the interference pattern has a minimum at $\beta = 2\pi$ and the fringe visibility V is therefore

$$V = 2AB/(A^2 + B^2) \quad (5)$$

The observation of the interference pattern exhausts the experimental possibilities for unpolarised neutrons.

Because there is no uncertainty relationship between spin and position it is possible, using polarised neutrons, to make simultaneous measurements of the interference pattern and of the neutron spin polarisation at each point along it. The additional information may be derived from the density operator ρ which according to equation (2) is transformed to

$$\rho_i(x) = M \rho_0(x) M^{-1} = \begin{bmatrix} \rho_{11}^0 & \rho_{12}^0 e^{-i\alpha} \\ \rho_{21}^0 e^{+i\alpha} & \rho_{22}^0 \end{bmatrix} \quad (6)$$

The polarisation P_i of the transmitted beam is defined by

$$P_i = \langle \sigma_i \rangle = \text{Tr}(\rho_i(x) \sigma_i) / \text{Tr}(\rho_i(x)) \quad (7)$$

and in particular

$$P_x = P_{x0} \quad (8a); \quad (P_x + iP_y)_i = (P_x + iP_y)_0 e^{i\alpha} \quad (8b)$$

The essential results are contained in equations (8) showing that although the component of the neutron polarisation in the recombined beam parallel to the magnetic field is unchanged from what it was before the division of amplitude, the polarisation vector in the plane transverse to the magnetic field is rotated through an angle α . Thus, although the density matrix contains sufficient information to allow us to make predictions about the absolute rotation of the neutron spins in the recombined beam it contains no information about the relative rotation of the spins in the constituent beams. The essential point is, of course, that whereas the phase difference β between the

spatially separated wave trains is determined by the mag. field and the passage time alone, the absolute angle of rotation α of the polarisation vector in the reconstituted beam also requires for its specification the mixing ratio B/A of the component wave trains as expressed through equation (4).

To emphasise the significance of these results from an experimental viewpoint we might envisage carrying out two limiting types of measurement. In the first case we set $A < B$ and determine the spin rotation of the transmitted neutrons which reaches the value π at $\cos \beta/2 = -A/B$, making the transition to 2π at $\beta = 2\pi$. Alternatively we can set $A > B$ in which case α achieves a maximum deviation of $2 \tan^{-1}[(B/A)/(1 - (B/A)^2)^{1/2}]$ at $\cos \beta/2 = -B/A$ and then drops rapidly back to zero at $\beta = 2\pi$. Thus the polarisation in the recombined beam carries out a rotation for $A < B$ and a nutation for $A > B$. There is no 'relative rotation'. When either A or B tends to zero the polarisation approximates to that of neutrons whose paths are constrained but the fringe visibility reduces in proportion.

At the other limit we set $A = B$ with unit fringe visibility and, as $\beta \rightarrow 2\pi$, we find that $\alpha \rightarrow \pi$ and the polarisation in the transmitted beam is reversed. Evidently there is a discontinuity in α at $\beta = 2\pi$ in the transition from $A < B$ to $A > B$ which means that at maximum fringe visibility the spin rotation becomes indeterminate at the interference minimum.

Apparently we must omit the case $A = B$, representing a fringe visibility of unity and total polarisation reversal, because in this situation the transfer matrix is null at the interference minimum and no neutrons are transmitted. However, this results from a defect in the formalism which uses plane wave states; in the more realistic wave packet description the factor $\cos \beta$ in equation (3) is replaced by the real part of, and the visibility $V < 1$ by the modulus of, the complex degree of coherence in the interfering wave trains, thus the reduction of the transfer matrix to singular form is avoided.

Bosons

The equivalent situation for bosons may be explored by treating the case of a spin 1 particle whose spin wave-function is characterised by three independent amplitudes a_+ , a_0 , a_- , corresponding to states with spin component $M = 1, 0$ or -1 along the magnetic field. The density matrix now has 9 elements which may be organised into subsets of 1, 3 and 5 elements which are the expectation values of spherical tensors of rank 0, 1 and 2 respectively^{9,10}. The first rank tensor moments are just the components of the spin polarisation.

$$P_x = (|a_+|^2 - |a_-|^2)/I \quad (9a)$$

$$P_x \pm iP_y = \langle a_+^* a_0 + a_-^* a_0^* \rangle / \sqrt{2} I \quad (9b)$$

where $I = |a_+|^2 + |a_0|^2 + |a_-|^2$ is the total intensity.

In the presence of a magnetic field the amplitudes $a_{\pm}$ are shifted in phase to $a_{\pm} e^{i\alpha/2}$ and it should be noted from equation (9) that the transverse polarisation is linear in these amplitudes and their complex conjugates. The amplitude a_0 is unaffected by the magnetic field and this is crucial to the analysis since it follows that the transverse polarisation in the recombined beam does not contain any interference terms derived from products of field-dependent amplitudes. For spin 1 particles, equations (8) therefore become

$$P_x = P_{x0} \cdot (A^2 + B^2 + 2AB \cos \beta) / T \quad (10a)$$

$$(P_x \pm iP_y)_i = (P_x \pm iP_y)_0 ((A+B) \cdot (A+B e^{i\alpha})) / T \quad (10b)$$

where in this case the transmission

$$T = A^2 + B^2 + 2AB \cos \beta + 2AB(1 - \cos \beta)(|a_0|^2/I)_0 \quad (11)$$

is sensitive to the degree of alignment $|a_0|^2/I$ in the beam incident on the interferometer.

Equation (10b) exhibits the fundamental feature that the term $(A+B) \cdot (A+B e^{i\alpha})$ may be represented as the incoherent sum of a fixed polarisation $(A+B)A$ and a rotating

196 $\sin(A+B)B e^{\pm i\theta}$ whereas for spin $\frac{1}{2}$ fermions there is (rotating) term derived from the coherent superposition of field dependent amplitudes. The weights $(A+B)A$ and $(A+B)B$ are identical with the weights A^2 and B^2 obtained by incoherently superimposing the constituent beams, for the case of maximum fringe visibility but this is an important point as the presence of the two terms allows us to give an operational meaning to the concept of relative rotation in the recombined beam whatever their relative magnitude.

To show that the concept of 'relative spin rotation' retains a meaning for bosons in the setting of an interference experiment we only have to examine the corresponding predictions for the two limiting experiments discussed above in the case of fermions. When $A \rightarrow 0$ and the fringe visibility becomes vanishingly small we find from equation (10) that the transmitted polarisation vector rotates through the angle β as expected. When $B \rightarrow 0$ there is no rotation and the angle β between the two orientations of the analysing axis of a filter giving maximum transmission for these two cases defines the relative rotation in the absence of interference. At the opposite extreme, where $A \approx B$ and the fringe visibility is maximum, there are two groups of neutrons, with relative intensities $(A+B)A$ and $(A+B)B$ respectively, that is those whose spins have suffered no deviation and those whose spins have rotated through the same angle β . We can again measure the relative rotation, the same relative rotation β as for the case of zero interference, by observing the two directions of the analysing axis of the filter which permits free passage to neutrons. This is quite different to the fermion case where there is transmission at a single angle $\alpha \neq \beta$ only and no method of measuring a relative rotation.

Not surprisingly we find from equation (11) that the intensity in the interference pattern for bosons reaches a maximum when $\beta = 2\pi$. The minima in the interference pattern occur at $\beta = \pm\pi$ and it is interesting that the transverse polarisation vanishes at the minima, when conditions are such as to maximise the fringe visibility.

To examine the behaviour of the vector polarisation does not exhaust the possibilities where spin 1 particles are concerned because the second rank tensor moments are also experimentally accessible quantities. Denoting these moments by $t_{1,M}$ (refs 9, 10) we find

$$\begin{aligned} (a) \quad t_{2,0} &= (1/\sqrt{2})(|a_+|^2 - 2|a_0|^2 + |a_-|^2) \\ (b) \quad t_{2,\pm 1} &= (\sqrt{3}/2)(a_+ a_0^* - a_-^* a_0) \\ (c) \quad t_{2,\pm 2} &= \sqrt{3} a_+^* a_- \end{aligned} \quad (12)$$

Clearly the magnetic field-dependence of the moments $t_{2,0\pm 1}$ is the same in essential respects as that of the moments $t_{1,0\pm 1}$ whose cartesian components are given in equation (9). The tensor moments $t_{2,\pm 2}$ behave quite differently in a magnetic field in that they are shifted in phase by $e^{\pm 2i\theta}$ when the amplitudes $a_{\pm}$ collect phase factors $e^{\pm i\theta}$. Therefore $t_{2,\pm 2}$ revert to their original values when $\beta = \pi$ and the amplitudes $a_{\pm}$ change sign. Thus $t_{2,\pm 2}$ are totally analogous to the components of the vector polarisation of a spinor field.

Sign reversal effects

Clearly then where 'sign reversal' effects are sought, the number of states available to the particles is not crucial, the important thing is that two of the participating states should be phase shifted in opposite directions by the application of a suitable perturbation. This has been demonstrated by Klemp¹¹ who observed sign reversal effects for transitions between the hyperfine levels of the diatomic molecule TIF. The experiments were carried out on a molecular beam resonance apparatus using the Ramsey separated oscillatory coil technique. Thus, although both sign reversal and interference effects were generated in spin space the underlying principle was the same as for neutron double beam interference.

The distinction between the results derived for spin $\frac{1}{2}$ and spin 1 particles in the case of the vector polarisation has been

traced to the existence in the latter case of a state, the $M=0$ state, whose amplitude is insensitive to the presence of the magnetic field. It follows, therefore, that the spin $\frac{1}{2}$ results are characteristic of all fermions, whereas the spin 1 results are characteristic of all bosons of non-vanishing spin for which an $M=0$ state exists. The single known boson excluded by this criterion is, of course, the photon for which the spin state can be defined only with respect to the momentum with the $M=0$ state disallowed because of the vanishing rest-mass.

In the neutron interferometer the magnetic field causes the neutron spins in one arm to alter their state from positive helicity to negative helicity and back again. A photon analogue would require the role of the magnetic field to be filled by an optical element which converts right circular polarisation to left circular polarisation and back again to right circular polarisation. Two half wave plates in series perform precisely these functions and a full wave plate introduces a phase difference of 2π between the polarisation states exactly as a magnetic field does for the neutron spin-states. Indeed Bernstein, who first thought of the neutron interference experiment³ specifically compares the action of the magnetic field to that of a birefringent medium.

The active element in the interference experiment proposed above is a uniaxial crystal with its optic axis normal to the direction of propagation of the beam. If a_x and a_y represent the components of the complex vector potential parallel and perpendicular respectively to the optic axis then the amplitudes $a_{\pm}$ for states with spin component $M = \pm 1$ along the momentum are

$$a_{\pm} = \mp(1/\sqrt{2})(a_x \mp ia_y) \quad (13)$$

Thus the circular polarisation P_c in the beam is given by

$$P_c = i(a_x a_y^* - a_x^* a_y)/I \quad (14)$$

where

$$I = \langle |a_x|^2 + |a_y|^2 \rangle \quad (15)$$

is the intensity.

The relationship between outgoing and incoming amplitudes in the light beam traversing the crystal may now be expressed as

$$\begin{pmatrix} a'_x \\ a'_y \end{pmatrix} = \begin{pmatrix} e^{i\delta_x} & 0 \\ 0 & e^{i\delta_y} \end{pmatrix} \begin{pmatrix} a_x \\ a_y \end{pmatrix} = e^{i\Delta/2} \begin{pmatrix} e^{i\delta/2} & 0 \\ 0 & e^{-i\delta/2} \end{pmatrix} \begin{pmatrix} a_x \\ a_y \end{pmatrix} \quad (16)$$

where

$$\begin{aligned} \delta_x &= \frac{2\pi}{\lambda} n_o z & \delta_y &= \frac{2\pi}{\lambda} n_E z \\ \Delta &= (\delta_x + \delta_y) & \delta &= (\delta_x - \delta_y) \end{aligned} \quad (17)$$

z is the path length in the crystal, and n_o and n_E are the ordinary and extraordinary indices of refraction.

It follows from equations (14) and (16) that, for a beam prepared in a state of pure circular polarisation P_c without ellipticity, the output circular polarisation P'_c is given by

$$P'_c = P_c \cos \delta \quad (18)$$

Thus, the circular polarisation is reversed when $\delta = \pi$, which described a half-wave plate. Two half-wave plates in series thus recover the original circular polarisation but change the signs of the amplitudes according to equation (16). The remainder of the theory proceeds as before and we can construct the analogue of the transfer matrix in equation (1) with β replaced by $-\delta$ and B by $B e^{i\Delta/2}$.

The observability of the photon amplitudes has no relevance here as we are concerned solely with observations on the Stokes operators¹² which characterise the polarisation properties of the beam and are quadratic in these amplitudes. The Stokes operators satisfy the same commutation rules as angular momenta and in view of the homomorphism of the group SU(2) on the group R3 any unitary transformation on a two

state system such as the photon has an analogous rotation for a spin $\frac{1}{2}$ fermion⁵. This justifies the photon analogue proposed above which is exact, provided the cycle of unitary transformations on the spin states of the photon is not interpreted as a rotation in real space. One can construct another photon analogue by using an optically active crystal to introduce a phase difference of 2π between the amplitudes $a_{\pm}$; in this case a real rotation of the plane of polarisation does take place but it is a rotation through an angle π rather than through an angle 2π (ref 4).

To observe the minimum in the optical interference pattern obtained by introducing a full wave plate in one arm of an interferometer it would be necessary to eliminate the phase factor $e^{i\Delta/2}$ in equation (16) by equalising the optical paths in both arms using a compensator according to normal optical practice. Two half wave plates in series with their optic axes at right angles would fulfil this role ideally. This requirement does not invalidate the principle of the device and indeed the same

defect exists in the neutron experiment (the 'residual λ shifts'³) although in a less severe form because the equivalent 'optical path difference' vanishes to first order provided the neutron energy is sufficiently low.

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Mass mortality of photosynthetic bacteria as a mechanism for dark lamina formation in sediments of the Black Sea

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Black Sea seasonal entrainment of dissolved oxygen below the chemocline results in a temporary hiatus in which an oxygen induced mass mortality of photosynthetic bacteria initiates their mass sedimentation. During their sedimentation black coloured FeS-rich granules form. The abundance of these granules in the sediments dictates their colour and microlamination pattern.

RHYTHMICALLY laminated sediments from meromictic lakes and seas have long fascinated geologists, limnologists and oceanographers as these lamina have been used to construct detailed chronologies based on the supposition that a light and dark coloured band (couplet) represents events which repeated themselves on an annual basis.

Most of the sediments of the Black Sea are microlaminated¹ and these microlamina are thought to represent annual events or varves². On this basis a detailed chronology has been erected for the sediments of the Black Sea^{3,4}. Discrepancies between the ¹⁴C age and the varve count age for some of the recent Black Sea sediments¹ prompted us to examine these same sediments. An hypothesis concerning a mechanism for varve formation was formulated to explain the relative lack of correlation between the ¹⁴C and varve count chronologies in these microlaminated coccolith-rich sediments.

Recent reports⁵ have speculated that during both Cambrian and Precambrian times the majority of the oceans and seas of the world were meromictic, that is, permanently stratified into a deep non-mixing anaerobic layer termed the monimolimnion, and an overlying oxygenated mixing layer termed the mixolimnion. These conditions are similar to those currently found in the meromictic Black Sea⁶. To this extent the Black Sea

serves as a 'living fossil' and an understanding of the conditions responsible for dark and light coloured lamina formation there should assist us in interpreting the microlaminated deposits in the sediments of Cambrian and Precambrian seas.

The Black Sea is the largest 'lake' in the world⁶. The history of its tectonically formed euxinic basin, which now contains essentially marine waters, has included long periods when the basin was occupied by relatively fresh water⁷. We have therefore adopted the terminology of Hutchinson⁶ in describing this meromictic body of water. The water masses of the present day Black Sea are divided by a sulphide-rich chemocline into a lower saline anaerobic zone (monimolimnion) and an overlying less saline seasonally stratified mixolimnion.

During the last thousand years, a carbonate-rich microlaminated coccolith ooze has been deposited in anaerobic conditions at depths of 200 m or more along the bottom of the Black Sea^{1,2}. The detailed chronology of the Black Sea sediments discussed above was first formulated in the early thirties and forties^{3,4}. Many sediment studies of the Black Sea have been carried out⁷ since then but none have hinted at the possible impact of a mass mortality of photosynthetic bacteria on the microlamination pattern within its sediments. These bacteria are one of the main primary producers within the Black Sea⁸ and their annual mass mortality was not suspected until it was observed in another much smaller meromictic body of water in Quebec, Canada⁹.

The present study attempts to prove that oxygen-induced mass mortality of these photosynthetic bacteria (which are restricted to the anaerobic waters below 160 m)¹⁰ has been a major factor contributing to dark lamina formation in the Black Sea. Information is presented to show that the seasonal mass mortality of these photosynthetic bacteria occurs as a result of oxygen entrainment into the anaerobic waters which they occupy and that this deep-layer oxygenation is most likely

Vertical profiles at station A for dissolved O₂, salinity, specific conductivity, water density and H₂S and bacterial abundance and biomass for stations A and B

Rep.	Depth (m)	Oxygen* (p.p.m.)	Salinity* (PPT)	Conductivity* (μs cm ⁻¹)	Temperature* (°C)	Density (sigma T)	H ₂ S	Bacterial† density at A	Bacterial‡ biomass at A	Bacterial density at B	Bacterial biomass at B
—	0.5	6.83	17.70	23.02	14.70	12.80	0	Photosynthetic bacteria were absent between 0 and 200 m		Photosynthetic bacteria were absent between 0 and 160 m	
—	10	7.26	17.90	23.04	14.72	12.95	0				
—	25	7.28	18.40	23.70	14.40	13.39	0				
—	50	8.40	18.10	21.40	9.80	13.88	0				
—	60	7.84	18.30	20.40	8.00	14.25	0				
—	75	6.50	18.90	20.36	7.40	14.78	0				
—	80	5.20	18.96	20.56	7.44	14.72	0				
—	85	3.60	19.20	20.90	7.44	15.01	0				
—	90	2.92	19.70	21.44	7.60	15.38	0				
—	100	2.18	20.00	21.90	7.90	15.59	0				
—	115	—	20.40	22.34	8.14	15.87	0				
—	125	0.61	20.54	22.46	8.65	15.92	0				
2	150	—	20.65	22.57	8.75	15.99	t§	0.7 (0.3/1.1)	0.35	1.2(0.8/1.6)	0.60
4	160	0.54	20.80	22.71	8.74	16.11	t§	0.6(0.2/0.9)	0.30	228(202/254)	114
3	165	0.54	20.86	22.77	8.79	16.15	t§	2.7(2.1/2.9)	1.35	1.8(1.2/2.1)	0.88
4	170	0.52	20.95	22.86	8.80	16.22	t§	8.2(6.7/11.2)	4.10	1.4(1.0/1.9)	0.71
2	175	0.38	21.00	22.91	8.87	16.25	t§	13.8(11.0/16.5)	6.9	1.2(0.9/1.5)	0.60
2	200	0.00	21.35	23.26	8.90	16.52	p§	160.0(11.8/20.3)	80	4.6(3.5/5.6)	2.30
2	225	0.00	21.70	23.60	8.97	16.78	p§	1.6(0.9/2.2)	0.8	1.3(0.7/1.8)	0.65

* Oxygen, salinity, specific conductivity, temperature and density were recorded only at station B. Bathythermograph results indicated a sharp thermocline beginning at 37 m (14 °C), 40 m (13 °C), 42 m (12 °C), 43 m (11 °C), 45 m (10 °C), 47 m (9 °C), 50 m (8.4 °C).

† Bacterial density at station A as numbers per ml times 10³ with the maximum and minimum values (× 10³) recorded in parentheses.

‡ Bacterial biomass at station A as mg m⁻³, calculated on the observed mean bacterial size of 0.5 μm³ per cell.

§ t, trace; p, present.

to occur during winter when partial destratification of the mixolimnion has occurred.

After the photosynthetic bacteria are killed by the addition of dissolved oxygen to their environment, they begin to settle slowly (1–2 m h⁻¹)¹¹ towards the bottom. During their descent the elemental sulphur which they store as a waste product of their H₂S-based photosynthesis¹² is exposed to the ferrous-rich waters of the upper layers of the anaerobic zone¹⁰. The elemental sulphur once exposed is capable of acting as nuclei for the formation of black ferrous sulphide compounds. The presence of ferrous sulphides and their products (such as FeS₂ pyrite) produces dark-coloured microlaminae.

Experimental methods

Using a Simrad SK3 sonar unit deployed in the Turkish hydrobiology research vessel, R/S Arar, it was possible to detect and later sample the thermocline and chemocline at 29° 05' E, 41° 40' N at a depth of 830 m, 30 km from the Bosphorus sill near Kilyos, Turkey (station A). Nansen bottles and a 10-l stainless steel Petersen styled closing cylinder were

used to remove replicated water samples for immediate preservation and later bacteriological analysis. A simple Van Dorn sampler replaced the more sophisticated equipment mentioned above at the Trabzon, Turkey Station (39° 55' E, 41° 10' N) which was located in 800 m of water (station B).

Quantitative estimates of algal and bacterial densities were made using a Fein Optik haemocytometer and a Leitz Orthoplan microscope. The plankton from immediately above and below the H₂S chemocline (160 m at station A, and 200 m at station B) was preserved with Lugol's IKI solution and concentrated by simple sedimentation¹³. Salinity, specific conductivity and temperature were measured at station B using a Beckman R-55-3 electrodeless salinometer. Dissolved oxygen was estimated at the same station using a Winkler calibrated Wissenschaftlich-Technisch Werkstätten Oxidigi Meter (model 5500). Such equipment was not available in Trabzon.

Sediments from the Black Sea sampled during the Glomar Challenger deep sea drilling operation² were available for study. Black Sea sediments were analysed by scanning electron microscopy: samples were freeze fractured, affixed to SEM

Table 2 Abundance of black granules in light and dark coloured microlamina from the Black Sea core

Code* no.	Sediment colour	Sediment type	Sediment depth (cm)	Ferrous sulphide† black granules [(no. per μg) × 10 ³]	Confidence‡ interval (P = 0.05)
57	Light grey	Coccoliths	12	47	904
28	Light beige	Coccoliths	15	52	1,409
21	Light grey	Coccoliths	22	23	1,005
11	Off white	Diatoms	41	135	1,604
14	Off white	Aragonite	62	200	1,704
30	Dark brown	Coccoliths	12	1700	60,023
73	Nearly black	Coccoliths	15	2400	90,012
13	Dark brown	Coccoliths	22	800	38,026
91	Dark brown	Diatoms	41	1200	10,024
65	Nearly black	Sapropel	62	3900	80,035

* Samples were coded to avoid unconscious bias of the counts.

† Mean granular densities per μg dry wt of sediment × 10³.

‡ Two standard deviations erected about the opaque granule densities were based on three replicates.

stubs and gold coated. Light- and dark-banded material from individual microlaminae was removed using fine dissecting forceps under a dissecting microscope to ensure that the material was not contaminated by adjacent lamina.

To determine the abundance (density) of dark coloured granules in the light and dark microlaminae, the previously isolated sediments were dried for 24 h at 80 °C and weighed after cooling. A sample of 0.1 mg from each of five light coloured lamina and five dark coloured lamina was suspended in 1.0 ml distilled water and a 0.1 ml aliquot of the homogenate was placed on an haemocytometer. The number of black granules were recorded for each of 40 randomly selected haemocytometer fields. A similar technique was used to determine the density of the photosynthetic bacteria from the IKI preserved water samples from the Black Sea.

Vertical distribution of photosynthetic bacteria in the Black Sea

As photosynthetic bacteria are light dependent, they develop population maxima at the minimal distance from the surface at which sulphide is available to them¹². In the Black Sea, sulphide is unavailable above a depth of 150 m at most locations¹⁴. Furthermore, these photosynthetic bacteria are killed by minute concentrations of oxygen¹⁵. The water column of the Black Sea is rarely anaerobic above a depth of 150 m (ref. 14).

On 31 October 1977 the photosynthetic bacteria were found concentrated at a depth of 200 m at the Kilyos station in the Black Sea (Table 1). Micrographs of these bacteria (Fig. 1a) indicated that the dominant form possessed an external morphology similar to that described by Buchanan and Gibbons¹⁵ for the photosynthetic bacterium *Thiocapsa roseopersicina* (spherical with a mean diameter of 1.5–2 µm). Many of our samples seem to contain blue-green algae as well as several other types of photosynthetic bacteria which we have yet to identify.

Individuals of *T. roseopersicina* in samples which were inadvertently exposed to small amounts of oxygen before preservation were often surrounded by a slime-filled envelope which may have caused them to aggregate into clumps (Fig. 1b). Irregularities in cell shape (Fig. 1a) were ascribed to the internal storage of elemental sulphur which this species produces photosynthetically as a waste product during the oxidation of H_2S ^{12,15}.

Photosynthetic bacteria taken from the other end of the Black Sea near Trabzon, Turkey on 17 November 1977 were similar to those at Station B but were concentrated at a slightly shallower depth (160 compared with 200 m) (Table 1).

Comparing our autumn photosynthetic bacterial densities with the winter densities reported by Kriss¹⁶ (for his Yalta-Batumi stations) it was evident that his February photosynthetic bacteria maximum was located at a much greater depth than ours. By late February the water at Kriss's winter stations was oxygenated to a depth of 300 m and the photosynthetic bacteria were concentrated at a depth of 500 m, indicating that oxygen might have been entrained down to that depth some time earlier or alternatively that the high bacterial densities which he observed at 500 m and deeper, may have included large numbers of dead bacteria which were settling out. Kriss's winter observations suggested that Black Sea waters below 200 m might be seasonally exposed to dissolved oxygen.

Grasshoff calculated that 500 km³ of surface waters containing an estimated 5×10^6 metric tons of oxygen penetrates the chemocline of the Black Sea each year¹⁴. Furthermore, Brewer *et al.*¹⁷ observed elemental sulphur as well as manganese and iron oxide granules in the anaerobic surficial sediments of the Black Sea along the Turkish (Anatolian) coast in 1,000 to 2,000 m of water. They interpreted this to mean that oxygen-rich surface waters had contacted the sulphide, manganese and iron-rich waters at and below the anaerobic chemocline in the Black Sea¹⁰ oxidising these waters and thereby precipitating elemental sulphur, iron and manganese oxides.¹⁷

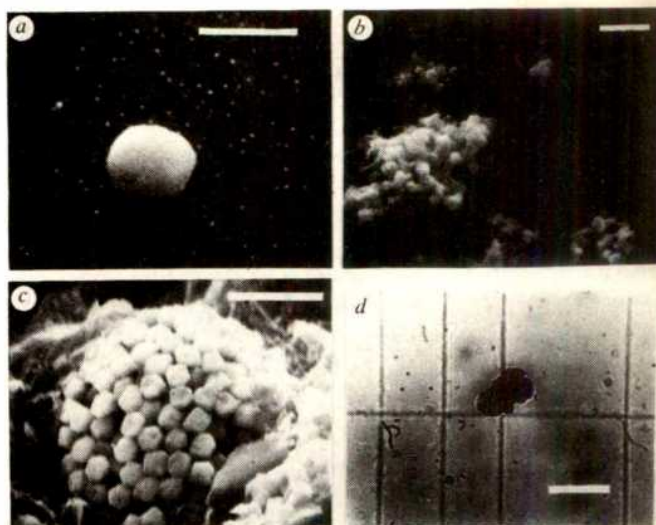


Fig. 1 a–c, SEM photographs. a, *Thiocapsa roseopersicina* from a depth of 160 m at the Trabzon, Turkey station. Irregularities in cell surface may be the result of the internal storage of elemental sulphur¹⁵. Background specks are crystals caused by the evaporation of Lugol's¹³ IKI preservative in which the original samples were stored. Scale bar, 2 µm. b, A colony of *T. roseopersicina* from 160 m at the Trabzon station which had been inadvertently exposed to dissolved oxygen before preservation. Cells are bound together by thick mucillagenous secretions. Scale bar, 10 µm. c, A pyrite (FeS_2) framboid from a black microlamina at 4 cm depth in a sediment core from the Glomar Challenger station 381 (ref. 5). Scale bar, 2 µm. d, A transmitted light microscope photograph of a pyrite granule from a depth of 260 m in the Black Sea near Kilyos, Turkey. Although its framboid structure was later verified using SEM, its possible resuspension cannot be ruled out. Scale bar, 5 µm.

Vertical mixing of oxygenated surface waters to depths of 300 m or more in the Black Sea seems to occur in winter when the surface waters have been cooled and the water column approaches homeothermy. In addition, increased surface salinity before late winter rains reduces the amplitude of the vertical salinity gradient¹⁸. It is not surprising, therefore, that Kriss¹⁶ observed dead photosynthetic bacteria in well oxygenated water above 100 m after heavy winter storm activity presumably swept them up from the chemocline.

These four lines of evidence from independent studies all indicate that a seasonal mass mortality of photosynthetic bacteria is the result of partial and temporary oxygenation of the upper anaerobic layers of the Black Sea in winter.

Figure 2 provides a schematic representation of these events. In the uppermost diagram, six zones are represented by the letters A–E. These symbols depict the following portions of the Black Sea's water column: (A) the warm-water upper mixolimnion; (B) the metalimnion, upper and lower boundaries of which are represented by the dashed lines; (C) the oxygenated cold-water portion of the mixolimnion; (D) the anaerobic portion of the mixolimnion; and (E) the permanently anaerobic monimolimnion. The 'dots' concentrated along the top of the anaerobic mixolimnion (zone D) represent the photosynthetic bacterial plate.

During winter (central diagram) the metalimnion disappears as the thermal gradient (not shown) becomes less intense as surface temperatures decrease¹⁸. Except for a relatively few photosynthetic bacteria (which secrete sufficient slime to form a bacterial mass (Fig. 1b) which is enough to prevent dissolved oxygen from reaching the core before the bacterial mass has settled into deeper anaerobic waters) the intrusion of oxygen into the previously anaerobic portion of the mixolimnion (zone D) results in the mass mortality of all the anaerobic bacteria in this zone. Subsequently, these dead bacteria settle towards the bottom of the Black Sea at a rate of between 1 and 2 m h⁻¹ (ref.

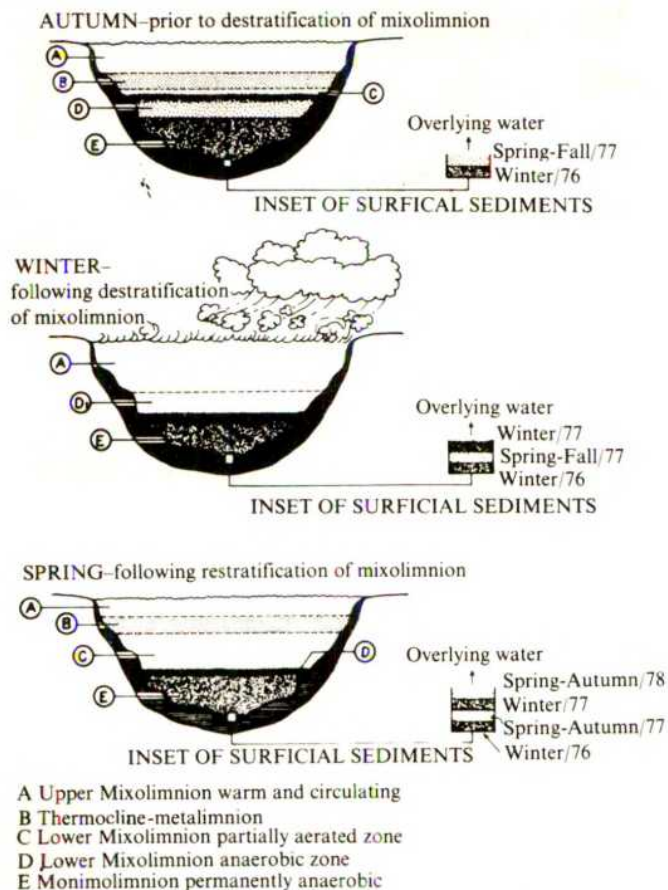


Fig. 2 Schematic representation of the major seasonal changes in the vertical distribution of planktonic photosynthetic bacteria in the Black Sea with insets depicting the formation of light and dark microlaminae (see text).

11). During their descent they serve as nuclei for the formation of black FeS-rich compounds. Many of these compounds later form raspberry shaped pyrites (FeS_2) called 'framboids' (Fig. 1c, d).

The bottom diagram in Fig. 2 depicts the water column of the Black Sea in early spring when the majority of the mixolimnion is still oxygen rich. As spring and summer pass, decaying organic matter (tripton) from the overlying water accumulates on or near the chemocline where its continued decomposition removes any dissolved oxygen from the overlying water mass. This process increases the thickness of the anaerobic region of the mixolimnion (zone D). This in turn permits the photosynthetic bacteria to move closer to the surface where light intensities are higher¹⁹. By early winter, most of the subthermocline mixolimnion has been deoxygenated and a condition similar to that depicted in the uppermost diagram of Fig. 2 exists. The process previously described then repeats itself if winds of sufficient intensity to mix oxygenated surface waters to the depth of the photosynthetic bacterial plate reoccur.

Dark lamina formation

Metal sulphides such as FeS are extremely insoluble in neutral or alkaline water such as that found in the Black Sea²⁰ ($\text{pH } 7.5 \pm 0.2$)²¹. Thus HS^- in the presence of ferrous ions will form FeS compounds which give anaerobic sediments their characteristic black colour²². However, the exact nature of the compounds that form is dependent on the presence and concentration of a number of additional species in solution such as carbonates, silicates and heavy metals²⁰.

In the conditions observed in the Black Sea (alkalinity 4.4 meq l^{-1} (ref. 21); H_2S (HS^-) concentration of $300\text{--}350 \mu\text{g-atom l}^{-1}$ (ref. 21); a temperature 7 and 9°C ¹⁸; salinity of 22 ppt ¹⁰; and depending upon the depth of water, pressure of up to 200 atm) the precipitation of iron monosulfides would be

expected to occur at a ferrous iron concentration of roughly $4 \times 10^{-7} \text{ mol l}^{-1}$ (ref. 20). Only the sulphide-rich chemocline in the Black Sea contains such high ferrous iron concentrations¹⁴. Iron sulphide granules have been previously described for the sediments of the Black Sea^{5,22}. The general consensus seems to be that some of these black granules such as the pyrites may form during early diagenesis²³ however, Degens² described these same framboids in water samples taken several hundred metres above the sediment-water interphase in the Black Sea. These observations suggest that framboid formation begins in the water column following the mass mortality of photosynthetic bacteria. Since most of the Black Sea Basin is $1,000 \text{ m}$ or more in depth these bacteria would not reach the bottom for nearly 50 d given a sedimentation rate of $1\text{--}2 \text{ m h}^{-1}$. Thus it may be possible for their mineralisation and subsequent pyritisation to occur during their descent.

Data presented in Table 2 indicate that when black granule densities exceed about 1×10^6 per μg dry wt of sediment, a dark layer can be expected to form in the recent (coccolith-rich) sediments of the Black Sea.

The term black granule has been used above to indicate any opaque mass viewed with transmitted light microscopy. We are currently working (with S. Haynes) to determine the chemical composition of these iron- and sulphur-rich black granules. Several polished plastic impregnated cross-sections from the microlaminated Black Sea surficial sediments ($0\text{--}34 \text{ cm}$) have been produced. Pyrite framboid concentrations in these polished sections were 2 to 3 times higher in the dark coloured microlamina than in the light coloured ones.

Our studies and those of others^{8,16} have indicated a massive accumulation of photosynthetic bacteria just below the sulphide-rich chemocline in the Black Sea. These obligate anaerobic bacteria are instantly killed by the introduction of dissolved oxygen into their immediate environment.⁹ Following winter storm activity, large numbers of these photosynthetic bacteria have been observed in the surface waters of the Black Sea,¹⁶ indicating that vertical mixing had taken place.

The death of the photosynthetic bacteria on exposure to dissolved oxygen results in their gradual mineralisation as they sink slowly towards the bottom. During this process of mineralisation, these sulphur-filled bacteria act as nuclei for ferrous sulphide framboid formation. These framboids enter the bottom sediments of the Black Sea along with other sedimenting particles such as diatoms, coccoliths and clastics. Depending on the final black granule density and the degree of 'whiteness' of the underlying layer, a dark microlamina may form as shown inset in Fig. 2. However, if storm events during any particular winter fail to generate winds capable of oxygenating the chemocline, bacterial mass mortality there will fail to occur. As a result, a dark-coloured microlamina may fail to form. Discrepancies between 'varve' count and ^{14}C chronologies in recent Black Sea sediments may in part be related to this phenomenon.

The history of the oceans is in part the story of the progressive oxidation of their primordial anoxic environment²³. Degens and Stoffers⁵ have related the evolution of the oceans to the recent history of the present-day stratified waters of the Black Sea. The sediments of this curious body of water bear a striking resemblance to those deposited during Cambrian and Precambrian times in the bottom of some of the then existent world oceans⁵.

Data presented here indicate that dark lamina formation in this meromictic body of water is intimately associated with the mass mortality and subsequent mineralisation of the sulphur-rich photosynthetic bacteria which colonise the chemocline in the Black Sea.

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letters to nature

High energy X-ray spectrum of Her X-1

OBSERVATIONS of Her X-1 taken during the on-state of its 35-d cycle¹ between 31 August and 12 September 1977 with the high-energy X-ray detector on board OSO 8 are reported here. A detailed analysis of the time-variable background spectrum suggests that the line features observed in our uncorrected time-averaged spectrum and those previously reported at 25, 60 and 135 keV can be caused by a $\sim 1\%$ change in detector gain. After correcting for this effect, a $3\text{-}\sigma$ upper limit of 4×10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$ is obtained on the line flux at 60 keV in the time-averaged spectrum, a flux significantly lower than that observed by Coe *et al.*² 6 months earlier. A $3\text{-}\sigma$ upper limit of 2×10^{-3} photon $\text{cm}^{-2} \text{s}^{-1}$ obtained for our pulsed spectrum at the same energy is higher than, and hence consistent with, the positive result reported by Trümper^{3,4} from a balloon flight during the same time interval.

The discovery by Trümper *et al.*^{5,6} of two strong lines in the pulsed X-ray spectrum of Her X-1 has inspired many other groups to search for this phenomenon. The stronger of the two lines, at 58 ± 5 keV, has now been observed by Trümper *et al.* on two separate balloon flights, one in May 1976⁵⁻⁷ and the second in September 1977^{3,4}. The second line, at an energy of approximately 110 keV, was observed in 1976 at the 2.2σ level, but in 1977 the line flux had decreased to below the detection sensitivity⁴. The measured absolute flux values for the two lines are given in Table 1. The credibility of these results is greatly enhanced by the observation that the flux in the lines pulses with the same 1.24-s period measured at lower energies. The time-averaged source spectrum can be distorted by systematic variations in the background spectrum or in the detector response. Because such variations are unlikely to occur with the same period as the observed pulsations from Her X-1, the use of the pulsed spectrum should eliminate these systematic effects.

No observation of Her X-1 by any other group has had the combination of sensitivity, energy resolution and time resolution necessary to positively detect and resolve either of these two lines. The reported line fluxes obtained by other groups at various different energies are given in Table 1. Unless otherwise indicated, the tabulated values are the line fluxes averaged over the 1.24-s period, or they are $3\text{-}\sigma$ upper limits. The standard deviation in the line fluxes are quoted directly from the respective authors and, in general, include the uncertainties in line width and instrument response function in addition to the statistical uncertainties. None of these reported line fluxes is

greater than 2.5σ although the statistical significance of the raw data of Coe *et al.*² is somewhat higher than this. Such a significance level is, however, reduced if the possibility of a line feature at any other energy is considered⁸. Recent results from the high-energy X-ray detector on HEAO 1 indicate a pulsed excess above the extrapolated low-energy spectrum consistent with the 1977 flux observed by Trümper *et al.* in the 58-keV line¹⁰.

The observations reported here were made with the high-energy X-ray detector on OSO 8, which is described in detail elsewhere¹¹. The instrument is an actively shielded CsI(Na) scintillation detector with a sensitive area of 27.5 cm^2 and a circular field of view with a full width at half maximum (FWHM) of 5° . The timing resolution is 0.3125 ms for each recorded photon, and the energy resolution is $\sim 25 \text{ keV}$ (FWHM) at

Fig. 1 The calculated counting rate spectrum of Her X-1 determined for the total observing time from 31 August to 12 September 1977. The counting rate is plotted on a linear scale to show the negative calculated fluxes at 45 keV, 70–100 keV, and $>170 \text{ keV}$ and also the apparent line features centred at $\sim 60 \text{ keV}$ and $\sim 135 \text{ keV}$.

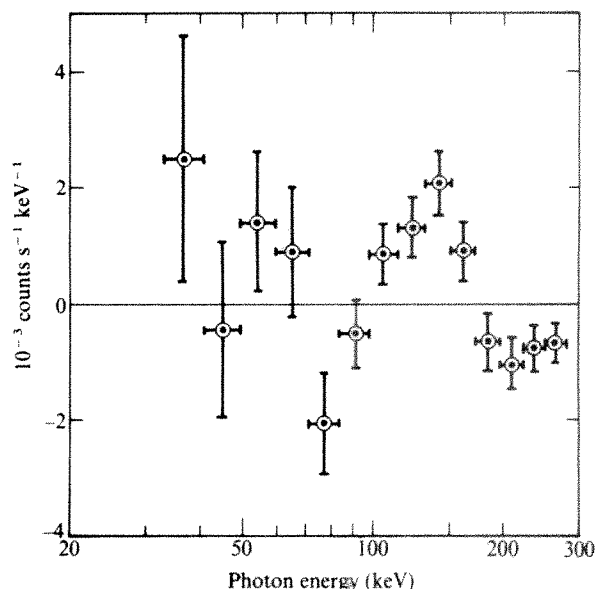


Table 1 Reported X-ray line fluxes from Her X-1 in photons cm⁻² s⁻¹

Date	Vehicle	Refs	25 keV	60 keV	110 keV	135 keV
May-June 1972	OSO 7	8	$(2.4 \pm 1.2) \times 10^{-2}$	—	—	—
15 May 1975	Balloon	9	—	$<6 \times 10^{-3}$	—	$<4 \times 10^{-3}$
24 June 1975	Balloon	15	—	$<2.6 \times 10^{-3}$	$<1.9 \times 10^{-3}$	—
3 May 1976	Balloon	5, 6	—	Pulsed $(2.9^{+3.0}_{-1.0}) \times 10^{-3}$	Pulsed $(1.4 \pm 0.6) \times 10^{-3}$	—
3-13 Feb 1977	Ariel 5	2	—	Pulsed $(1.7 \pm 0.7) \times 10^{-2}$	$(1.7 \pm 0.6) \times 10^{-3}$ *	—
3 Sept 1977	Balloon	4	—	$(1.1 \pm 0.1) \times 10^{-3}$	$<10^{-3}$	—
31 Aug- 12 Sept 1977	OSO 8 (proportional counter)	Pravdo (personal communication)	—	Pulsed $<9 \times 10^{-3}$	—	—
	OSO 8	This paper	—	$<4 \times 10^{-3}$	—	$<2 \times 10^{-3}$
	Csl (Na)	time-averaged This paper pulsed	—	$<2 \times 10^{-3}$ Pulsed	—	$<1 \times 10^{-3}$ Pulsed

* M. J. Coe, personal communication.

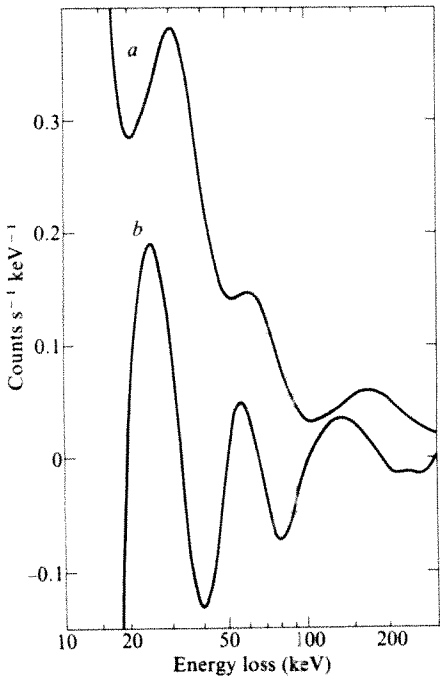
60 keV. The instrument is mounted in the rotating wheel section of OSO 8 with its field of view offset from the anti-spin axis by 5°. During the Her X-1 observations, the anti-spin axis of the spacecraft was orientated at an angle of $(5 \pm 0.5)^\circ$ from the direction to the source thus causing a modulation of the source signal for each wheel rotation. Our method of analysis uses this 10-s modulation to determine an accurate time-averaged source spectrum in the presence of the rather large and variable detector background¹². The pulsed spectrum is determined by combining the on-source data modulo the source period taken from Pravdo *et al.*¹³. The photon detection times are first adjusted for the time-of-flight variations between the source and the satellite.

The time-averaged spectrum obtained from all of our Her X-1 data shown in Fig. 1 contains two significant line features, one at ~60 keV and the other at ~135 keV. These features are not evident, however, in the pulsed spectrum. Furthermore, the

calculated source fluxes at 45 keV, 80 keV and >180 keV were found to be negative. We now believe that the apparent line features and the negative source fluxes are all caused by a small change in the gain of the photomultipliers at each wheel rotation. Such a change in gain, of the order of 1% or less, can result from the changing orientation of the photomultipliers (RCA 31016F) in the Earth's magnetic field. A cylinder of 4-mil CO-NETIC magnetic shielding (Perfection Mica Co.) was put around each photomultiplier, but a residual variation of gain with magnetic field remains. This is presumably because it was not possible to extend the magnetic shield one diameter beyond the photocathode as recommended by RCA. The effect of such a change in gain on the detector counting rate depends on the shape of the background spectrum. Our background spectrum is shown in Fig. 2 together with the difference between two background spectra measured with a gain difference of 1%. Such a difference spectrum is the apparent source spectrum which would be calculated in the absence of any real source. The line features and the negative source strengths in the time-averaged Her X-1 spectrum clearly match this difference spectrum both in energy and in the relative amplitudes of the peaks and valleys. The possibility⁸ of a third positive feature at 25 keV is interesting although, in our measurements, such an excess is lost in the statistics of the real source flux at that energy.

Assuming that a small gain change accounts for the features in the source spectrum, it is easy to compute the true time-averaged spectrum. This is possible in spite of the complicated variations in the strength and direction of the Earth's magnetic field seen by the photomultipliers as the satellite moves in its orbit. The shift in background spectrum resulting from the gain change at each wheel rotation always results in an apparent source spectrum equal to the difference spectrum shown in Fig. 2 multiplied by some factor which can be either positive or negative. This is true only when the shape of the background spectrum remains constant. This is satisfied to the required accuracy using only data collected well out of the South Atlantic Anomaly. The true source spectrum is determined by subtracting the background difference spectrum, for some mean value of the gain change, from the calculated source spectrum. The mean value of the gain change can be most easily determined by a least-squares technique in which the assumed gain change is adjusted until the calculated source strengths in all energy bins are positive or zero to within the statistical uncertainties. The time-averaged spectrum obtained in this way and unfolded through the detector response function using the methods of Dolan *et al.*¹² is shown in Fig. 3. Only data from the non-eclipsed parts of the four binary periods during which the source flux was

Fig. 2 a, Detector background spectrum for the Her X-1 observing time. b, The difference ($\times 25$) between two background spectra measured with a gain difference of 1%.



highest (31 August–6 September 1977) were used to obtain this spectrum. No significant features are evident above the 3σ levels given in Table 1. Positive detection of the pulsed flux was made up to an energy of 33 keV and no significant features are observed in the pulsed spectrum above the 3σ upper limits given in Table 1.

An understanding of features observed in our time-averaged spectrum of Her X-1 has been demonstrated in terms of small changes in gain of our X-ray detector. Such changes in gain can produce apparent line features in the spectrum at energies which have previously been reported in the literature. Brecher and Ulmer⁸ have pointed out the probable relationship to the detector background of the 1.5σ feature at 25 keV in the OSO 7 spectrum of Her X-1. Durouchoux *et al.*⁹ have shown that a small change in gain can explain the apparent line features at 57.5 and 137.5 keV in their time-averaged Her X-1 spectrum obtained on a balloon flight in May 1975. Coe *et al.*² report a line feature at 64 ± 6 keV in the time-averaged Her X-1 spectrum

from the high-energy X-ray detector on Ariel V. Attempts to explain their result in terms of a gain change have not been successful because the detector background spectrum does not have the required shape¹⁴.

The upper limits on the line features in our pulsed spectrum of Her X-1 are consistent with the results of Trümper *et al.*³⁻⁷. They do show that the line features are not significantly stronger when averaged over a full binary period as compared to the flux measured during a relatively short balloon flight. Finally, it must be emphasised that the reported line at 58 keV remains highly credible in view of its pulsed nature. Confirmation by different observers and measurements with improved energy resolution to determine the true line width remain highly desirable objectives.

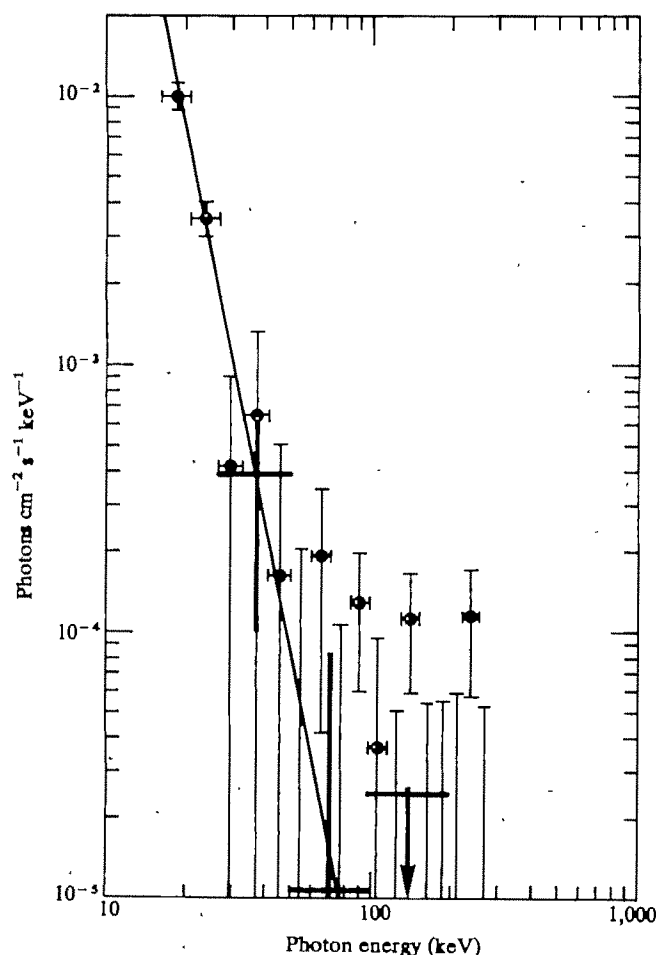
We thank Roger Thomas, the OSO Project Scientist, for his efforts in making this long observation of Her X-1 possible, and Steve Pravdo for supplying us with the observational parameters of the pulsations. G.S.M. was supported by NASA grant NSG 5066 and E.P.C. by contract NAS5-24451.

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Fig. 3 The time-averaged spectrum of Her X-1 for the period 31 August to 6 September 1977, during which the intensity was at its highest level. The count-rate spectrum for this period was corrected for an average change in gain of 0.7% and unfolded through the detection efficiency, K-escape probability, and detector resolution using the method of Dolan *et al.*¹² The best fit power law indicated is of the form

$$(2.4 \pm 0.4) \times 10^{-3} (E/25)^{-2.9 \pm 0.7} \text{ photons cm}^{-2} \text{ s}^{-1} \text{ keV}^{-1}.$$

The circled points are for energy bins each the width of the detector resolution (1σ) at that energy. None of the points in the spectrum deviate from the power-law fit by more than 2σ . The heavy lines show the source flux averaged over the wider energy intervals indicated. The vertical lines indicate 1σ statistical uncertainties or 1σ upper limits.



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Physical reality of shot noise model for the short-term variability of Cyg X-1

TERRELL¹ pointed out that the rapid intensity variation of Cyg X-1, first discovered in Uhuru observations², was well simulated by shot noise, that is, random superposition of shot pulses of constant shape ($\Sigma h(t-t_i)$; t_i is a random variable). Since then this model has become widely accepted and used to summarise the observational data³⁻⁷ which suggest the physical process of overlapping bursts of X-ray emission from local hot spots in an accretion disk around a black hole. The variability characteristics of shot noise are roughly determined by two parameters⁸, shot rate λ and effective pulse length τ_{eff} of a single shot $h(t)$. Almost all the observational data so far

obtained seem to be consistent with shot noise having a pulse length of a fraction of a second—although shot rate varies from one observation to another—which is thought to support the validity of the model. This letter points out that shot noise is only an example—and a rather artificial one—of a random process which can explain the basic observed properties of the variation (its correlation time and magnitude) and why it does not need to be singled out among many types of possible stochastic processes. Therefore, physical interpretation of the observation strictly based on the shot noise parameters may be misleading.

First note that a stochastic time series $x(t)$ is described by a set of statistics—the first order one $\langle x(t) \rangle$ (average), the second order one $\langle x(t_i)x(t_j) \rangle$ (variance and autocorrelation), the third order one $\langle x(t_i)x(t_j)x(t_k) \rangle$ and so on. A major concern in shot noise analyses has been to estimate the two parameters τ_{eff} and λ from observational data. Note, however, that shot noise can be completely assimilated to observational data by adjusting $h(t)$ so that its autocorrelation equals that of the data. The shot rate λ is a scaling factor for the magnitude of variation. The shot noise generated this way gives a complete simulation of the data up to the second order statistics. In other words, for any given time series a shot noise can always be generated so that there is no way to distinguish them within the second order statistics.

Such an all-inclusive nature of shot noise obscures the physical meaning of the shot noise parameters, especially λ if τ_{eff} has model-independent meaning of a characteristic time scale. Its applicability to data does not prove the physical reality that X rays are indeed emitted in pulses with shot rate λ , without other additional supporting facts, observational or theoretical. Shot noise was originally introduced to explain and analyse the fluctuation of electric current in vacuum tubes, which is justified because it is essentially a quantum effect: with Cyg X-1 there is no such justification.

Sutherland *et al.*⁹ have discussed an extended shot noise model in which steady component was taken into account, introducing another parameter, 'shot noise fraction' f . This discussion shows that λ and f cannot be uniquely determined by the second order statistics, and, in fact, the third moment $\langle x^3(t) \rangle$ was used to estimate the parameters. The analysis not only involves the third moment whose estimation error can be rather large, but also depends on the assumed shape of $h(t)$.

The usefulness of such an analysis is examined in the following example. One of the most basic and physically important stochastic processes, is brownian motion, its simplest model is described by Langevin equation¹⁰,

$$\frac{du}{dt} = -\gamma u + R(t)/m$$

where u and m are velocity and mass of a brownian particle respectively, γ is coefficient of friction, and R is random force acting on the particle which can usually be considered to have a white spectrum (infinitesimal correlation time). The resulting stochastic time series $u(t)$ may be similar, in a generalised sense, to shot noise; fluctuations of u are generated by R and decay with $1/\gamma$, that is $h(t) = h \exp(-\gamma t)$ except that h in this case is not a constant but follows Maxwellian distribution law. It is possible to apply the extended shot noise model forcibly to $u(t)$, but the best fit parameters λ and f will hardly have any physical meaning, and indicate still less the collision rate with surrounding particles and 'shot fraction'. Should we try to apply the model introducing still higher moments to deduce the distribution parameters of h ? The physically relevant part in this example is the correlation time $1/\gamma$ of the fluctuation and the magnitude of variance $\langle u^2(t) \rangle$ which is equal to kT/m (equipartition law), and higher order statistics are of secondary importance.

As illustrated by the above example (and relaxation and fluctuation theories in statistical physics), physical understand-

ing of variation should be sought from the second order statistics, before getting too deeply involved in a rigid model based on *ad hoc* assumptions. Assuming shot noise with $h(t)$ and λ defines the complete set of statistics (or the probability density) which is practically beyond observational tests. Whether variation is shot noise or gaussian noise or any other type of fluctuation does not affect the second order statistics; physical discussion is possible without such a specification.

Finally, there are observational indications in Cyg X-1 which do not favour the simple shot-noise view of overlapping unit bursts. Ogawara *et al.*¹¹ noted the energy dependence of the variation of the rocket data in the 1.5–25 keV range and discussed the compatibility with the shot-noise view. Recently I carried out a balloon observation¹² of the short-term ($\sim$ s) variability in the hard (≥ 20 keV) X-ray region with a large-area detector, to obtain an integral, wide-band picture of the variation. These results, together with those of the former rocket observation¹¹, suggest that the variation is essentially spectral, keeping the total X-ray luminosity relatively constant, although a narrow band observation indicates intensity variation. A possible model of such a variation based on the inverse-Compton emission from a two-temperature disc around a black hole^{13,14} was suggested, which may exemplify a physical discussion noncommittal to the higher order statistics. The detail of the observation and discussion will be published elsewhere.

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The spectrum of the supernova in MCG43223

A POSSIBLE SUPERNOVA in the galaxy MCG43223 was found by Gilmore¹ on 8 May 1978. The complex emission and absorption features of its spectrum which we have recorded are described below. Our findings confirm that this object is a supernova of Type V.

Gilmore reported that, at the time of discovery, the object was ~ 17.5 mag and that it was not visible on plates taken on 8 March and 8 April. Its presence was confirmed² by taking a plate of the galaxy on 25 May using the UK 1.2 m Schmidt telescope, Siding

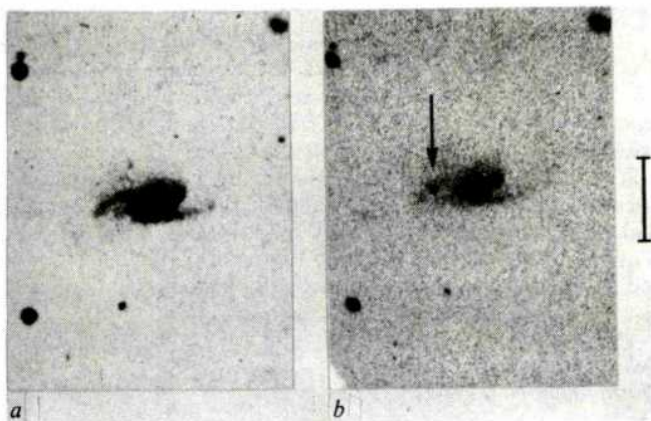


Fig. 1 Galaxy MCG43223: *a*, 60-min exposure on IIIaJ/GG395 filter 28 June 1976; *b*, 15-min exposure on IIIaJ/GG395 filter 25 May 1978. The scale is shown by the bar which is 20 arc s in length. North is at the top and east to the right.

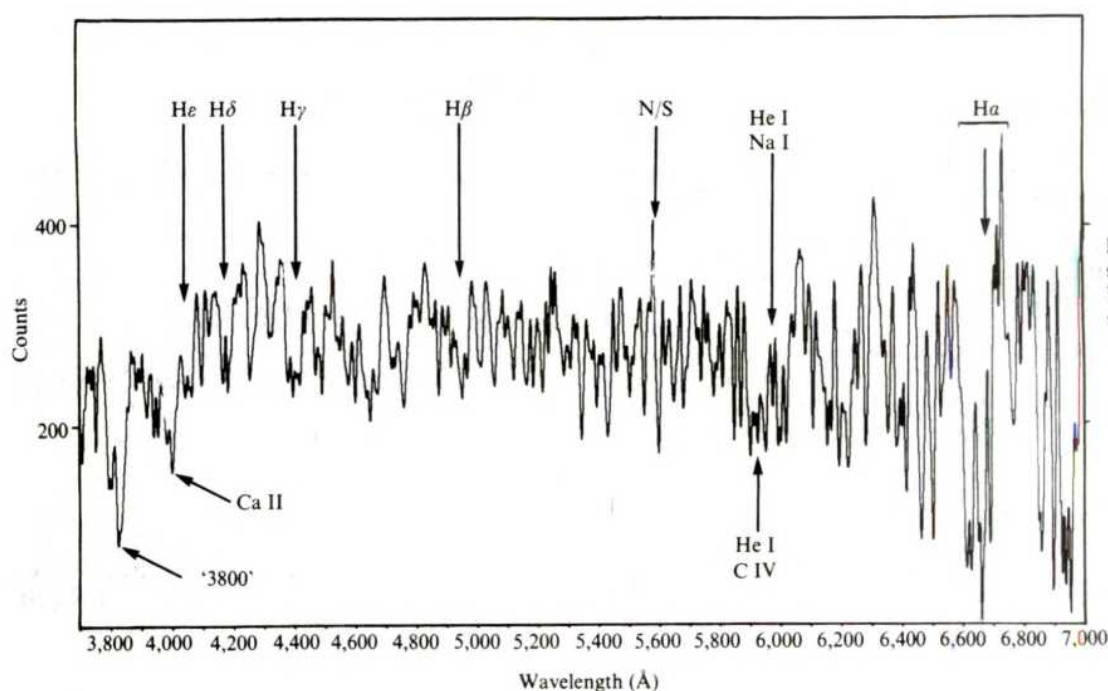


Fig. 2 The spectrum of the supernova in MCG43223: N/S refers to incomplete subtraction of the 5,577 Å night skyline.

Spring. The object's position was determined² to be RA = 13 h 27 min 32.60 s, dec = -21° 29' 24.0" (1950), being located 15 arc s due west of the galaxy's nucleus. Its photographic magnitude was estimated to be 20.0. A print taken from the UK Schmidt plate is shown in Fig. 1 where it is compared with a prediscovery photograph. Inspection of the print indicates that the object seems to be situated in a spiral arm of MCG43223. K.H.E. and J.C.B. obtained a spectrum of this object on 29 May with the Boller and Chivens spectrograph and Robinson Wampler³ image dissector scanner at the $f/15$ focus of the 3.9 m Anglo-Australian Telescope, Siding Spring. The spectrograph gave a nominal dispersion of 200 Å mm^{-1} , and a slit width of 2 arc s was used which corresponded to a final spectral resolution of $\sim 10 \text{ Å}$. The spectrum, which has been corrected for non-uniformity of the detector but not for its spectral response, is presented in Fig. 2. Bad weather severely curtailed our intended programme; we were unable to obtain standard star spectra or a spectrum of the galaxy.

We believe this spectrum, which shows a complex mixture of emission and absorption features, confirms that the object is a supernova in MCG43223. We have identified five features in the spectrum with H I, namely the Balmer series from H α through

to H ϵ ; and we tentatively identify He I, C IV and Ca II. In addition the spectrum contains an absorption feature near $\lambda(\text{rest}) = 3,800 \text{ Å}$ which seems to be a common feature in supernovae spectra⁴.

Comparing our data with the spectra illustrated by Greenstein and Minkowski⁴, we identify the supernova as Type V (a subclass of Type II), as it shows many features in common with the Type II 1961 V in NGC1058⁴. This is the first time that a spectrum of a Type V has been recorded so close to maximum. However, there is some difficulty in making the comparison, as the published data for NGC1058 refer to a period ~ 173 d after maximum. For our data the spectrum must have been obtained between 21 and 50 d after maximum. A survey of plates taken of this field between 5 April 1978 and a month later should give a more exact timing of the maximum. The Balmer lines give $z = 0.012$. Equating this redshift with that of MCG43223 we obtain a distance of 65 Mpc, using $H = 55 \text{ km s}^{-1} \text{ Mpc}^{-1}$. With the estimated brightness of $V \sim 17$ we obtain $M_v = -17.1$ assuming no absorption, which is reasonable considering the high galactic latitude ($b = 40^\circ$). This absolute magnitude compares well with the accepted value for Type II supernovae,

$M_v = -17.54 \pm 0.26$ given by Tammann⁵. We intend carrying out further spectroscopy on the object, providing that it does not fade too quickly, to monitor spectral variations.

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Search for the 1.2 MeV γ -ray line of Walraven and Haymes

WALRAVEN AND HAYMES¹ have reported the possible detection of a ~ 1.16 MeV γ -ray line at a flux level of $(3.4 \pm 1.5) \times 10^{-3}$ photons $\text{cm}^{-2} \text{s}^{-1}$ from a point in the galactic plane ($l = 345^\circ$, $b = 5^\circ$). If correct this could be an enormously important result constituting the first direct confirmation of theories of explosive nucleosynthesis (ref. 2 and refs therein). Clayton *et al.*^{3,4} have predicted that the most intense γ -ray line to be emitted from the radioactive debris of a typical supernova after ~ 3 yr will be the 1.156 MeV line from the decay of ^{44}Ti ($\tau_{1/2} = 48$ yr). However, the reported detection was at a marginal level of statistical significance (2.6σ). It was mainly the "remarkable double coincidence that just the theoretically-predicted spectral anomaly occurred" when the telescope was pointed at a region of the galactic plane believed to contain young supernova remnants, that encouraged these workers to report this result. Clearly an independent attempt to confirm this observation with a telescope of finer energy resolution is called for. Here we report the result of such an attempt. Our conclusion is that the reported positive detection was probably a statistical fluctuation.

A balloon-borne high-resolution (3.2 keV FWHM at 1.156 MeV) γ -ray telescope was flown on 11 November 1977 from Alice Springs, Australia. The instrument was essentially the same as that previously described^{5,6}, but an important modification made for this flight was the replacement of the central Ge(Li) detector with a large volume, $\sim 130 \text{ cm}^3$ high-purity germanium detector. This necessitated an increase of the effective entrance aperture in the 400 lb NaI anticoincidence shield to 16° FWHM at 1.156 MeV. The direction ($l = 345^\circ$, $b = 5^\circ$) was tracked for 1 h starting at 23.51 UT with the balloon at an atmospheric depth of 3.0 g cm^{-2} . The search for the 1.156 MeV line was terminated when our quick-look data revealed that no indication of the feature at the reported flux level was evident.

In Fig. 1 we show the sum of all our data in the vicinity of 1.16 MeV. Only corrections for drifts in the energy calibration with time have been made. No astrophysical γ -ray line is apparent in these data nor in any of our data within hundreds of keV of 1.156 MeV. These data can be used to set a 1.156 MeV γ -ray line flux limit. Using the known detector efficiency, instrumental dead time and atmospheric attenuation we find a

2σ limit of less than $2.8 \times 10^{-4} \sqrt{\text{width (in keV)}}$ photons $\text{cm}^{-2} \text{s}^{-1}$. Since the linewidth is expected to be ~ 40 keV (FWHM) (ref. 7) this corresponds to a 2σ limit of less than 1.8×10^{-3} photons $\text{cm}^{-2} \text{s}^{-1}$ which is a factor of two less than the reported intensity. Thus the reported positive detection seems likely to be a statistical fluctuation.

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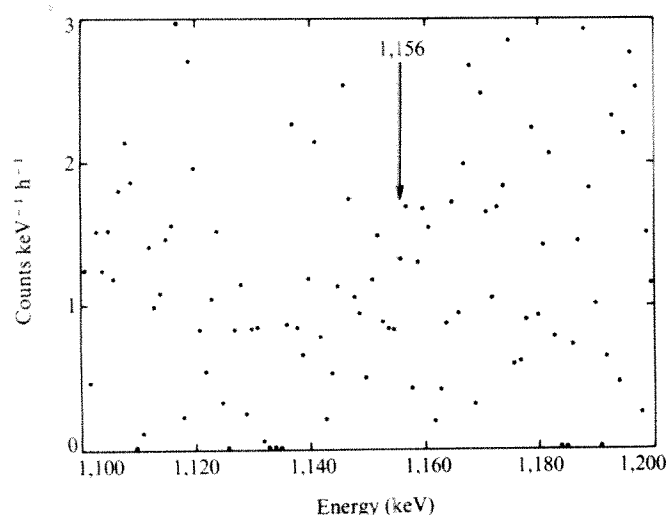
Does the troposphere respond to day-to-day changes in solar magnetic field?

WILCOX ET AL.¹ have reported a statistical correlation between the solar magnetic sector structure and the vorticity area index (VAI) during the period 1964–70. Characteristically, the VAI reached a minimum 1 day after the passage of a solar magnetic sector boundary (SSB), with the response being greatest in winter and at 500 and 300 mbar. This result has been the basis of many subsequent studies; in particular Wilcox *et al.*² strengthened their previous results by extending the period of analysis to the 11 year interval 1963–73. We have examined the period 1974–77 and find that this characteristic response is now no longer evident.

Williams has recently³ analysed the four components of the Lorenz energy cycle in an attempt to clarify the physical processes involved in the apparent solar modulation of the VAI. Although no statistically significant solar effects were discovered, some similarity was found in the behaviour of the eddy kinetic energy (KE) and the VAI. However, the KE exhibited a greater averaged response to the passage of an SSB (as obtained using a superposed epoch analysis) during the 1960s than the 1970s. Consequently, if there is a correlation between the KE and the VAI we might expect the effect of an SSB crossing on the VAI to also be weaker during the more recent years. This prompted us to extend the analysis to the period 1974–77 to determine whether or not the characteristic response of the VAI still existed during these years.

The VAI was calculated by the method described previously¹ using the National Meteorological Center data available at the National Center for Atmospheric Research. The VAI, at a particular pressure level, is defined to be that area of the Northern Hemisphere north of 20°N in which the absolute vorticity exceeds $24 \times 10^{-5} \text{ s}^{-1}$ plus that area in which it exceeds $20 \times 10^{-5} \text{ s}^{-1}$. This is a modification of the original index defined by Roberts and Olson⁴. In order to match the previous work, a superimposed epoch analysis was performed using the VAI and SSBs⁵ during the winter months 1 November–31 March. However, we required that all the VAI data be in this interval

Fig. 1 Energy spectra in the vicinity of 1.156 MeV. Raw data corrected only for drifts in the energy calibration. Each point represents a 1-keV wide energy bin.



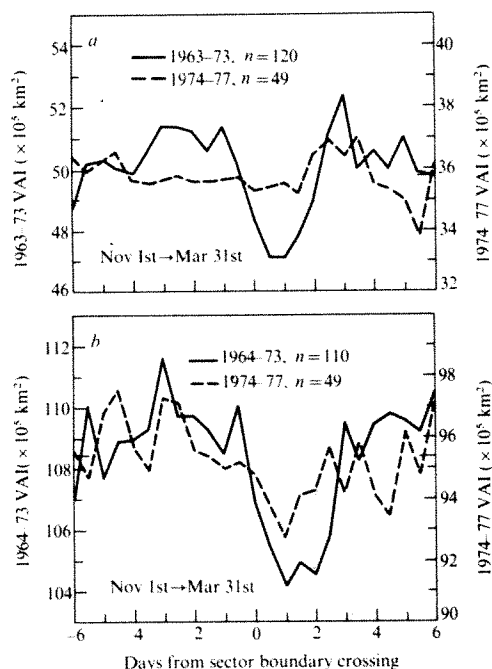


Fig. 1 Results of superposed epoch analysis of VAI plotted against SSBs during the periods indicated. *n*, no of SSB's included. *a*, 500 mbar; *b*, 300 mbar.

whereas the criterion used by Wilcox *et al.*¹ was that all the SSBs be in it. Our requirement eliminates 11 key days used by Wilcox² and 1 during the interval 1974-77. In all other aspects our approach is identical to that of Wilcox *et al.*¹.

Figure 1*a* illustrates the results at 500 mbar. For the interval 1963-73 we obtained results consistent with those of previous authors. This satisfied us that we were using the same analysis techniques as had been used in the earlier studies. However, for the years 1974-77 (our analysis ended in March 1977 so that 3½ years are included) we find a response completely unlike that of the previous years. Similarly, at 300 mbar Fig. 1*b* shows that although the absolute minimum is still on day +1 the magnitude of this response, at best, is greatly reduced. We thus find evidence to support the hypothesis of a correlation between the VAI and the KE.

In order to interpret this result we sought differences between the two time intervals which might qualitatively explain our findings. First, although the new result is for a short time interval, the number of key dates analysed, 49, is comparable to the 54 first analysed between 1964-70. Second, it may be argued that the years 1974-77 spanned a solar minimum so that we may expect little solar effect on the weather. This hypothesis was tested by examining a similar 3½ year period, 1963-66, which covered the previous solar minimum. However, during these years the VAI clearly exhibited the characteristic response to the passage of the SSBs². It therefore seems unlikely that the new result was caused by a lack of solar activity. Third, since a superimposed epoch analysis is essentially a cross correlation

integral, the results depend on any periodicities in either dataset. Thus, in this study, any differences in the spectrum of separation of key dates would influence the results. However, no obvious differences are evident in the histograms of sector widths as shown in Fig. 2. Fourth, it is clear from Fig. 1 that the typical values of the VAI are much lower during the more recent years. This suggests the possibility that the atmosphere is not in a 'suitable state' to respond to a solar impulse when the VAI is low. Finally, there is the possibility that the years 1963-73 or 1974-77 are yielding an anomalous result. Neither of these possibilities may be discounted using the current database.

As this is a purely statistical study there is no method for conclusively proving which, if any, of the last three possibilities is the correct interpretation. However, since there are, at present, no workable physical mechanisms, it seems that the simplest interpretation is that the years 1963-73 yielded an anomalous result which was of unusually high statistical significance⁶ and which was self-consistent for an unusually long time. We therefore conclude that our work weakens the evidence for this particular sun-weather correlation.

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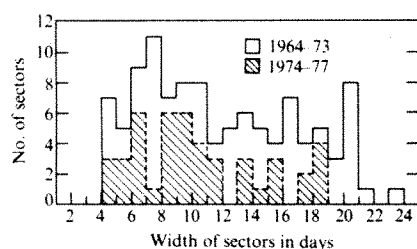
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Ordered arrangements of spheres of two different sizes in opal

GEM OPALS consist of a regularly ordered array of silica spheres of uniform size¹. Generally in any given gem stone the uniformity is maintained throughout the gem, although occasional samples contain bands in which the size varies from band to band. A sample of Brazilian gem opal of this banded type has now been found to contain a band in which there are spheres of two different sizes mixed together. Long-range ordering within this mixture has produced several different types of regular superstructures, which can be seen in section in electron micrographs of replicas of chips of this specimen (Fig. 1).

Packing arrangements of monodispersed spherical particles are of great general interest, and suspended latex spheres may order under the influence of electrostatic interaction from the surface double layers². Close-packed ordering also achieves a higher density than random packing, and it is generally conceded, but not proved, that a system of an infinite number of uniformly-sized spheres achieves its maximum density by regular close-packing³. The monodispersed spheres of silica in gem opal have been found to be packed in a close-packed manner⁴. Arrangements of mixtures of two sizes of sphere have been investigated experimentally⁵ and by computer simulation⁶ in order to determine packing densities, and to find out how the

Fig. 2 Histograms of width of sectors analysed in each period.



packing arrangement of one component is modified by the introduction of a second component of different size. No long-range ordering of the two components was observed in any of these cases, despite vibration of the arrays to simulate annealing⁵.

The regular arrangements of the two sizes of sphere in this unusual Brazilian sample were found in a number of replica fragments of a few chips from a single sample. The specimens used for electron microscopy were casually fractured, and so exposed surfaces of random orientation with respect to the array, with the orientation varying from fragment to fragment. The pieces of replica could not be related back to the original specimen. The appearance in an electron micrograph of a section through an array is expected to depend very much on the orientation of the section, but several different sections from this specimen could be recognised as coming from the same arrangement, and different ordered arrangements could be distinguished. Two approximate structures (to be described elsewhere) have been deduced from electron micrographs.

It appears that in this part of the opal there are just two sizes of sphere, with diameters of 0.18 and 0.1 μm respectively. In those places where ordering was observed it was most extensive, covering, for example, most of the area of a fragment of replica on a grid, for example, 1–2 mm. The different structural arrangements seem to be related to variations of the proportions of the two sizes of sphere in different places. Some places contained disordered mixtures of the two sizes, and in one place there was a boundary across which small spheres appeared to have penetrated into an array of larger ones. An example of part of the exposed surface of one of the ordered arrangements is shown in Fig. 1: it shows large spheres regularly dispersed among small ones, and close inspection shows that there is also a complex ordered arrangement of the small ones. How did the mixture of two sizes of spheres form and what was the driving force for long-range ordering?

It has been suggested⁷ that uniformity of sphere size in natural gem opal resulted from very slow, steady, near equilibrium conditions for the precipitation of silica from an underground solution. It is not clear what creates close-packing in the natural system. However, in laboratory experiments, monodispersed silica spheres settle quickly to a layer at the bottom of a vessel, and order (as shown by the formation of columnar grains exhibiting iridescence) increases over a period of months.

It seems likely that an understanding of the reasons for the ordering of the two sphere sizes found in the Brazilian sample might shed new light on the mechanism of opal formation. We have considered two possible mechanisms which could lead to ordering in the Brazilian sample. First, the two sizes of silica spheres might have formed concurrently and ordered whilst in

the colloidal state under the influence of long range double layer repulsions. This order could then be retained during sedimentation and compaction. Alternatively, ordering might have occurred solely under the influence of the Earth's gravitational field during compaction. This allows an alternative possibility of the two sphere sizes forming at different times and mixing by infiltration of the smaller spheres after an initial settling.

The first possibility is based on the knowledge that colloids consisting of spherical particles of equal size can order under the influence of repulsive double layer forces⁸. The question here arises as to what sort of ordering would be favoured by a colloid containing particles of two sizes. Would colloid theory predict a two-phase system with one phase containing small particles and the other phase containing large particles, or would a single mixed phase be preferred? A partial answer to this question has been obtained by considering pairwise potentials arising from van der Waals⁹ and double-layer forces¹⁰ between large spheres (U_{AA}), small spheres (U_{BB}), and mixed, large and small spheres (U_{AB}). For the simplest case of a system with equal numbers of small and large spheres we can simply regard the system as analogous to a binary alloy (see, for example, ref. 11). If then we find that $U_{AA} + U_{BB} > 2U_{AB}$, we conclude that a single mixed particle phase will result. Our calculations based on the observed sizes show that for systems with reasonably large double-layer interactions the above inequality is valid, down to a separation of the surfaces of the particles of about 50 Å, indicating that colloidal ordering could be responsible for the type of structures observed. As interparticle separations become less than 50 Å van der Waals forces become increasingly important and finally cause the particles to cohere strongly. At some later stage evaporation of the parent solution precipitates a final residue of silica which forms a cement and imparts additional strength to the opal. Gravity may play a part in compaction through sedimentation during opal formation in this model but is not essential to the ordering process.

The second ordering mechanism is one in which gravity is the controlling influence. One difficulty with the colloid model is in understanding how two quite distinct particle sizes could have been formed concurrently. In the gravity-controlled model we suppose that the smaller particles were formed after the larger ones, and penetrated into an already settled, but not yet compacted array of large spheres. Laboratory observations have confirmed that this process is possible for latex spheres¹². In these conditions sufficiently close contact would be maintained that pairwise interactions would be dominated by van der Waals forces. Our calculation shows that these forces alone do not favour the formation of a single phase, and therefore gravity must provide the ordering potential in this case. It follows that the stable system should be the one with the highest density. The density of the single phase would then be required to be higher than that of a close-packed monodispersed system, and this is certainly the case for one of the structures observed in Brazilian opal.

We conclude that it is not yet possible to determine which of the two suggested mechanisms of ordering occurs in the natural growth of opal. The fact that both major driving forces lead to a preferred AB (single phase) structure means that we are unable to use the two sphere size regions of the Brazilian gem to distinguish between the mechanisms. In nature both mechanisms may in fact operate. Their relative influences will depend on the precise physical and chemical characteristics of the parent solution, for example density, pH and ionic concentration.

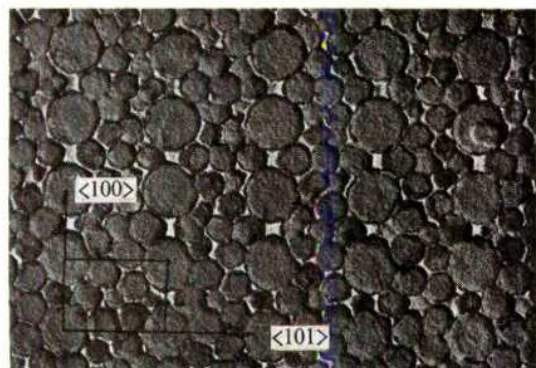
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Fig. 1 A small area of extensively ordered array of spheres of silica of two different sizes. A unit cell of surface structure is marked. The axes have been determined by relating this micrograph to sections found in other micrographs. The arrangement of small spheres varies slightly across the field of view because the section is slightly inclined to an axis.



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Anisotropy of melting for cubic metals

KINETIC studies of superheating of metals^{1,2} have shown that surfaces play an important part in initiating melting. Recent work on the anisotropy of vapour pressure³ suggests a strong dependence of melting on crystallographic orientation. There are models of melting⁴⁻⁷ which indicate that surfaces may become disordered (that is, lose their long-range order) at temperatures below the bulk-melting point. These temperatures are characteristics of the crystallographic planes of a metal. Based on simple considerations, we attempt here to estimate the melting temperatures of major low-index planes for close-packed cubic metals. The results are discussed in terms of low-energy electron diffraction (LEED) study and current theories of melting.

On the basis of Lindemann's melting criterion⁵, one can express in terms of harmonic approximation the relation between Debye temperature θ_D , melting point T_M , atomic weight M and mean-square amplitude of atomic vibration $\langle u^2 \rangle$ as⁸

$$\theta_D^2 = C \langle u^2 \rangle^{-1} [T_M/M] \quad (1)$$

where C , a constant, is equal to $9\hbar^2/k_B$ with $\hbar$ denoting the reduced Planck's constant and k_B the Boltzmann's constant. It has been well established through both experimental measurements and theoretical calculations that $\langle u^2 \rangle$ is much larger at a surface than in the bulk⁹. Consequently, the degree of anharmonicity should be much greater at the surface than in the bulk. From the various methods of studying surface anharmonicity¹⁰, the simple force constant method, which is based on anharmonic perturbation theory, is used here. Only the normal component of mean-square displacement denoted by $\langle u_z^2 \rangle$ is considered, as it is this parameter which is generally greater than the tangential component. The ratio of this value for surface (S) and bulk (B) that is, $\langle u_z^2 \rangle_S / \langle u_z^2 \rangle_B$ for various crystallographic planes, is only known for close-packed cubic metals as 1.87, 1.83 and 1.86 for (100), (110) and (111) planes, respectively¹¹. An analysis of equation (1) in terms of surface and bulk components followed by rearrangement would give

$$T_{MS}(hkl) = [\langle u_z^2 \rangle_S / \langle u_z^2 \rangle_B] [\theta_{DS}(hkl) / \theta_{DB}]^2 T_{MB} \quad (2)$$

A subsequent estimation of $T_{MS}(hkl)$ for planes (hkl) is presented in Table 1 for metals for which the various parameters of equation (2) are known¹²⁻¹⁴.

The present results on $T_{MS}(hkl)$ are considered to be fairly reasonable. For example, the surface melting temperature for Pb, which varies from (111) to (110) planes over a range of 49 K, is comparable with the 40 K obtained from LEED studies⁷. It is, of course, evident from equation (2) that T_{MS} values would depend on the choice of the ratio $\langle u_z^2 \rangle_S / \langle u_z^2 \rangle_B$ and on the adequate calculation of surface Debye temperature $\theta_{DS}(hkl)$ which is primarily based on summation of Morse potentials¹².

It has been pointed out¹⁵ that Lindemann's melting hypothesis consistently predicts the absolute melting temperature too high. It is argued in terms of amplitude of vibration of surface atoms that the 'critical amplitude' (for melting) will be reached at the surface before the interior. This view has been confirmed by LEED results⁷ and molecular dynamics studies⁴. The results in Table 1, with T_{MB} being consistently higher than T_{MS} , therefore agree with the above concept. It is also evident from Table 1 that T_{MS} decreases from (111) to (110) planes. Such a trend

Table 1 Some thermal properties of close-packed cubic metals

Metal	Bulk component		Surface component					
	Debye temperature* θ_{DB} (K)	Melting point† T_{MB} (K)	Debye temperature‡ θ_{DS} (K)			Melting point§ T_{MS} (K)		
			(110)	(100)	(111)	(110)	(100)	(111)
Ag	226.5	1,233	142	142	147	887	906	966
Cu	334.4	1,356	191	192	196	809	836	866
Ni	476.0	1,728	225	225	230	706	722	750
Pb	105.3	600	54	56	58	289	317	338
Pt	238.4	2,042	167	166	174	1,837	1,851	2,023

*Ref. 13. †Ref. 14. ‡Ref. 12.

§Estimated using equation (2).

apparently indicates the dependence of melting point on the density of packing of atoms in a particular crystal plane. One can thus conclude that a maximum vibrational instability is associated with the least densely packed plane. This conclusion is consistent with the theories of melting^{5,16,17}. Following the work of Maradudin *et al.*⁹, one would expect the amplitude of atomic vibrations to be maximal for planes with the least atomic packing density. It is known that the Debye temperature is closely related to the amplitude of vibration. As a result, θ_{DS} is expected to be lower with less closely packed planes; this is apparent in Jackson's results¹². Because, according to Lindemann's criterion, the melting point of a metal is directly proportional to the Debye temperature (equation 1), the lowest melting point is expected for a plane with the lowest surface Debye temperature, that is, with least close packing of atoms, as indeed has been indicated in the present work. On the basis of the estimated values of Table 1, a satisfactory quantitative relation has been obtained between $T_{MS}(hkl)$ and work function of the single crystal faces of a bare metal possessing close-packed cubic structure¹⁸.

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Superheated liquids containing suspended particles

THE superheat temperature limits of particle-free liquids have been previously investigated, notably by Skripov¹, Blander *et al.*², Apfel³ and Porteous⁴. Examinations of the homogeneous nucleation of liquid pentane have found the experimental limit of superheat temperature to be 146.5°C, which agrees well with the theoretical limit of superheat of 147.7°C calculated by Blander *et al.*² using the theories of Döring⁵, Volmer⁶ and Kagan⁷. Studies of superheating pure liquids have stressed the

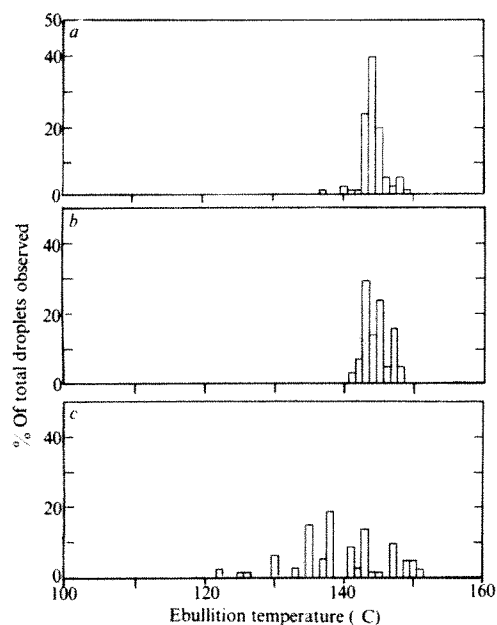


Fig. 1 *a*, Alumina (0.06 μm), run 3-15A; *b*, sericite (50 μm), run 3-14AC; *c*, carbon black (0.47 μm), run 6-12A. Particles suspended in pentane.

importance of keeping the liquid phase scrupulously clean and noted that the presence of ‘motes’ and particles interfered with obtaining experimentally consistent results because these served as nucleation centres; however, we know of no previous explicit study on the effects of suspended particles. We now report that liquid pentane and pentane/chloroform mixtures containing suspended solid hydrophobic and hydrophilic particles (carbon black, alumina, sericite) have been superheated as high as 110°C above their normal boiling points, and to within 3°C of the theoretical homogeneous limit of superheat of the particle-free liquid. This is the highest degree of superheat ever reported for liquids in the presence of solids other than smooth glass capillaries.

We examined the superheating of liquid *n*-pentane containing various amounts of suspended carbon black (Cabot; MT-X25, 0.47 μm diameter, Sterling R, 0.075 μm), alumina (Rhode Island Abrasives, 0.05 μm) and the mineral sericite

[(OH)₂KAl₂(AlSi₃O₁₀)] (50 μm). We used a method of superheating liquids modified from that of Defour⁸, who superheated droplets of test liquid suspended in an immiscible, less volatile, host liquid, that acted as a ‘liquid skinned’ vessel, thereby avoiding contact with a solid container wall. We used a thermal gradient column modelled after those used by Moore⁹, Skripov¹⁰, Apfel³, Blander *et al.*² and Porteous⁴.

Pentane droplets (~1 mm diameter) were heated above their normal boiling point (36.1 °C) as they ascended slowly in a 47-mm i.d. glass column filled with ethylene glycol (host liquid), in which a vertical temperature gradient existed. The gradient section of the column was heated electrically with a spiral winding of nichrome wire. The spiral pitch decreased from 2 cm at the lower end of the column to 0.5 cm near the top. The temperature gradient ranged over 4.0–1.5 °C cm⁻¹ in the zone of superheat nucleations. The lower section of the column was unheated to permit calculation of the droplet diameter from its rate of rise. Temperatures at which nucleation occurred (explosively) were measured with thermocouples immersed in the host liquid at the level of nucleation.

Solid particles were suspended in the pentane by stirring. The suspensions were unstable and were stirred immediately before use. Pentane/chloroform solutions were also used because the polar chloroform improved the stability of the suspensions of carbon black. We found that superheat limits of the particle-free pentane/chloroform solutions were in approximate agreement with the molar average superheat limits of the pure components¹¹, and that the effect of carbon particles on the superheat limit of the mixed solvent was generally less than on the pure solvent. The chloroform not only stabilised the carbon but inhibited its nucleation propensity.

Our primary results are summarised in Table 1 and Fig. 1; the latter shows histograms of the percentage of total nucleation events against temperature. The nucleation temperatures for alumina (Fig. 1*a*) and sericite suspensions (Fig. 1*b*) are narrowly distributed, with means at 144.1 °C and 145.4 °C, respectively. The superheats attained with carbon black suspensions are lower (139.4 °C) and more broadly distributed, probably because of adsorption of gas on the carbon particles. Figure 1*c* is a histogram for a carbon black/pentane suspension (run 6-12A) which was refluxed for 18 h to drive off entrapped gas before injection into the superheating column. The deviation of nucleation temperatures is narrower, and the mean temperature of nucleation higher than for carbon suspensions with shorter pretreatment times.

Table 1 Superheating of suspensions

		Particle	Run no.	Soak time	Soak temperature (°C)	Mean particle diameter (μm)	Bulk volume % solids in test droplet	No. of observations	Mean ebullition temperature (°C)	Standard deviation
50/50 vol. % pentane/chloroform		None	4-3F	—	—	—	—	34	156.4	3.07
		Carbon black (Sterling R)	4-6AG	1 h	24	0.075	0.6	28	155.1	2.69
		Carbon black (MT-X25)	4-3E	1 h	24	0.47	4.0	32	153.8	2.20
		Carbon black (MT-X25)	4-4DI	1 h	24	0.47	6.0	32	150.5	3.81
100 vol. % pentane		None	3-6A	—	—	—	—	69	146.5	0.96
		Sericite	3-14AC	1 h	24	50.2	14.0	72	145.4	1.76
		Alumina	3-15A	1 h	24	0.06	12.0	108	144.1	1.57
		Carbon black (MT-X25)	3-15B	1 min	24	0.47	10.0	41	110.2	10.1
			3-11E	15 min	24			121	126.5	10.7
			3-11D	1 h	24			136	131.4	8.4
			3-11C	3 h	24			94	132.4	8.82
			2-28	1 h	31.6			143	133.0	4.50
			6-12A	18 h	31.6			80	139.4	6.53

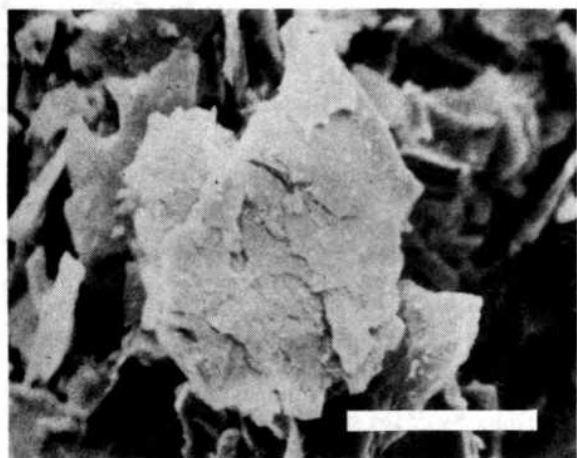


Fig. 2 Electron micrograph of sericite particle. Scale bar = 40 μm .

Carbon black particles are hydrophobic, whereas the alumina and sericite are hydrophilic. The consequent different wettabilities do not explain the observed superheat limit differences, that is, that the hydrophilic particles show a higher limit than the hydrophobic.

In general, the results are consistent with the nucleation theory of Volmer⁶, as extended by Cole¹², Jarvis¹³ and Eberhart¹⁴. The theoretical effect of interfacial energies on the limit of superheat would be that a change in wetting angle from 0° to >90° changes the calculated limits of superheat by only 2–3 °C. The difference in the superheat limits observed for the carbon suspensions and the inorganic suspensions must therefore reside in the gas adsorption capacity of the former.

In spite of the agreement with nucleation theory, the results may seem counter-intuitive because we tend to think of a particle as a discontinuity in the liquid phase and, hence, a point where phase separation is likely to occur. The careful filtration and cleaning of fluids by other investigators would seem to have had little effect on their experimental results other than removing particles that contained entrapped gas.

Previous studies of superheated liquids in contact with solid surfaces other than freshly drawn glass or silica capillaries^{15–18} failed to produce superheats of pentane in excess of 10 °C, whereas this study produced superheats of 74–110 °C. That our surfaces are not smooth is evident from the electron micrograph shown in Fig. 2. Nevertheless, our liquid–solid interfaces suffer the initiation of a vapour phase about as reluctantly as does homogeneous liquid.

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The problem of the thrown string

ULAM¹ reports that "there was another problem which Fermi wanted to study but which we somehow never came to formulate well or to work on. He said one day, 'it would be interesting to do something purely kinematical. Imagine a chain consisting of very many links, rigid, but free to rotate around each other. It would be curious to see what shapes the chain would assume when it was thrown on a table, by studying purely the effects of the initial energy and constraints, no forces'." Sygne² posed the problem of estimating the average $\bar{D}$ of the rectilinear distance D between the ends of a flexible string of length L when thrown down at random on a table. Later³ he published conceptual models and some experiments which together increased the challenge (recently restated by Kingman⁴): the models yielded values of $\bar{D}/L$ equal either to 0 or 1 unity, whereas in three experimental series by different throwers of different strings, the values obtained were 0.32 (100 throws), 0.34 (50 throws) and 0.38 (60 throws). (Only for the last of these was the value of $\bar{D}^2/L^2$ also recorded, at 0.18.) Sygne wrote³: "the agreement of the results is rather striking and one might hope to explain the numerical value (say 1/3) on theoretical grounds . . . I hoped that someone would suggest a way of treating directly a system with an infinite number of degrees of freedom, but perhaps no such way exists". Here we propose a solution along the desired lines by kinematical means.

Consider first the thrown string before it touches the table. Supposing that the rectilinear distance between its ends is x , how are the configurations of the string constrained by its continuity and its length? No element of the string can lie outside the ellipsoid having semi-axes $\frac{1}{2}L$, $\frac{1}{2}(L^2 - x^2)^{1/2}$, $\frac{1}{2}(L^2 - x^2)^{1/2}$, with the ends of the string at its foci; and any point within the ellipsoid may be intersected by the string. Thus the continuous nature of the string associates the accessible volume $\pi L(L^2 - x^2)/6$ with each value of x . We make this feature of the problem central to our treatment, and postulate that the probability density of x is proportional to the associated accessible volume. According to this postulate, if a large number N of throws is made, the number of cases for which the separation distance lies in the interval $(x, x + dx)$ is $3N(L^2 - x^2)dx/2L^3$, the proportionality factor being fixed by normalisation.

Next we introduce the vector $\mathbf{x}$ (of length x) from the lower to the higher end of the string, at the moment of impact of the string with the table, and model the effect on the separation distance of the collapse of the string onto the (horizontal) table, by projecting $\mathbf{x}$ on to the plane. Let θ be the smaller angle between $\mathbf{x}$ and the vertical. Assuming that the throws do not generate preferred values of θ , we adopt an isotropic distribution of (upwards) $\mathbf{x}$ -directions, and seek the distribution of projected lengths $D = x \sin \theta$. Consider the hemispherical surface of radius x lying flat on the table: its element belonging to the interval $(\theta, \theta + d\theta)$ contains the fraction $2\pi(x \sin \theta)(x d\theta)/2\pi x^2 (= \sin \theta d\theta)$ of the vertices of the vectors $\mathbf{x}$ drawn from the centre.

Then the number out of N throws for which, at impact with the table, the separation distance lies in $(x, x + dx)$ and the orientation angle in $(\theta, \theta + d\theta)$, is given by

$$n(x, \theta) dx d\theta = \frac{3N(L^2 - x^2)}{2L^3} \sin \theta d\theta dx \quad (1)$$

To obtain the observed distribution of the projections $D = x \sin \theta$, we change the independent variables x, θ in equation (1)

to x , D (Jacobian $(x^2 - D^2)^{-1/2}$), and integrate over x :

$$n(D) dD = \frac{3ND}{2L^3} \left[\int_D^L (L^2/x - x)(x^2 - D^2)^{-1/2} dx \right] dD \quad (2)$$

$$= \left[\frac{3N}{2L} \arccos(D/L) - \frac{3ND}{2L^3} (L^2 - D^2)^{1/2} \right] dD$$

Hence the probability density $\omega(\lambda)$ for the ratio $\lambda = D/L$ is

$$\omega(\lambda) = \frac{3}{2} [\arccos \lambda - \lambda(1 - \lambda^2)^{1/2}] \quad (3)$$

and for the density $\nu(u)$ of $u = D^2/L^2$ we find

$$\nu(u) = \frac{\omega(u^{1/2})}{2u^{1/2}}$$

Both ω and ν are convex and monotonically decreasing in the interval $(0, 1)$. While ω falls with only slight curvature from $3\pi/4$ to zero, ν falls from infinity to zero.

From equation (3) we obtain by straightforward integration the numerical values of the observables: $\bar{\lambda} = \bar{D}/L = 3\pi/32 \approx 0.29$, $\lambda^2 = D^2/L^2 = 2/15 \approx 0.13$. Considering the simplicity of our probabilistic postulate, these values compare favourably with the observations quoted above. At the conventional 0.05 significance level, there is no significant difference between the theoretical variance 0.046 and the observed variance 0.036, nor between the theoretical mean and the observed means in two of the experimental series. However, the differences of the theoretical mean from the largest observed mean, and from the mean of the pooled observations, are significant.

If the string was thrown so as to keep it in a plane parallel to the table throughout, the counterpart of ω would be constructed by replacing the ellipsoid by an ellipse, with the results $\bar{D}/L = 4/3\pi \approx 0.42$, $D^2/L^2 = 0.25$. We note that all the observed means lie between those calculated from the ellipsoid and from the ellipse, and the observed mean square is correspondingly bracketed. This may be because (some of) the throws were made near the table and along it.

How does the above analysis apply in the case of a segment of length L belonging to a longer string? In this case our original postulate might require non-trivial modification to take account of the presence of extraneous bodies, namely the extra portions of string. The problem of the thrown string has points of contact with properties of coiling macromolecules in solution, especially when approached as a limit of a chain^{3,5}. However, systematic results for the former problem depend on persisting effects of the randomisation of initial conditions, whereas for the coiling molecules results depend on the more special state of thermodynamic equilibrium, independent of initial conditions.

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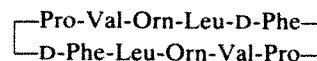
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The crystal structure of a hydrated gramicidin S-urea complex

THE cyclic decapeptide antibiotic gramicidin S has the sequence



Since its isolation by Gause and Brazhnikova¹, several authors have proposed possible conformations²⁻¹⁰. Of these models, the one that has gained most general acceptance is that of Hodgkin and Oughton², who proposed that the structure consisted of two β -chains linked by proline residues and stabilised by four intramolecular hydrogen bonds. Several derivatives of gramicidin S have been crystallised^{8,11}, but it had not been possible to solve any of the structures¹². We now report the structure of a gramicidin S-urea complex in which the gramicidin molecule has a conformation somewhat distorted from the Hodgkin-Oughton model.

In the presence of urea and HCl, gramicidin S crystallises as yellow elongated prisms from a solution in 95% ethanol. Crystal data: trigonal, $a = 25.8$, $c = 21.49$ Å, space group $P3_121$, $D_m = 1.10$, $D_c = 1.06$ (based on $C_{60}N_{12}O_{10}H_{92} \cdot \frac{1}{2}[(NH_2)_2CO] \cdot 8H_2O$, $Z = 6$). Data collected on a Hilger and Watts Y290 4-circle diffractometer consisted of 5,695 observed reflections out of 6,353 measured. Because of crystal instability it was necessary to use several crystals during data collection.

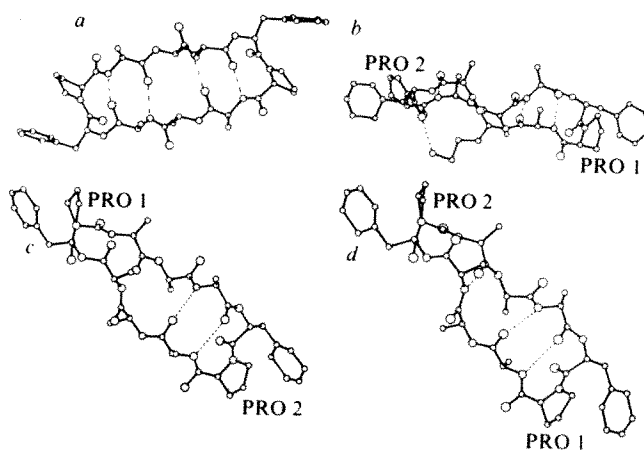


Fig. 1 Most of the side chains are omitted from the diagrams for the sake of clarity. *a*, A view of the molecule showing the four inter-chain hydrogen bonds, and the essential similarity of the structure to that proposed by Hodgkin and Oughton. *b*, A view showing the twist in the molecular backbone, and the unpredicted hydrogen bond involving the ornithine side chain. *c*, *d*, Two views related to each other by a rotation of approximately 180° , showing that the molecular backbone has approximate 2-fold symmetry.

The structure was solved by use of the multiple-solution direct methods program MULTAN¹³ developed in this laboratory. 100 phase sets were generated for 350 reflections ($E_{\min} = 1.86$), using 4,897 triple-phase invariants. The starting set consisted of one origin-defining and six other reflections (one with restricted phase and five with general phases). The phase set with the greatest combined figure of merit corresponded to an E -map which contained a 58-atom molecular fragment, and subsequent weighted E -maps revealed the remainder of the molecule and one half-molecule of urea.

The atomic coordinates and isotropic temperature factors were refined by the diagonal least-squares method, using fast Fourier transform techniques to generate structure factors and

gradients¹⁴. Refinement based on the 4,902 observed reflections within the sphere of 1.0 Å resolution gave an *R*-factor of 0.188, with approximate estimated standard deviations for the positions of carbon atoms within the main chain of 0.04 Å. The isotropic temperature factors (*B*) ranged from 5.0 to 25.1 Å². Because of the high thermal motion and the limited quality of the data, there is some uncertainty in the positions but not the presence of some of the side-chain atoms.

The molecular structure is basically similar to that of the model proposed by Hodgkin and Oughton². Figure 1a shows the four hydrogen bonds predicted from the model. The molecular backbone, however, is twisted from that of the model in such a way as to retain the approximate 2-fold symmetry but so that the hydrogen bonds between the two halves of the molecule are no longer parallel (Fig. 1b–d). In addition to these four intermolecular hydrogen bonds, there is an intramolecular hydrogen bond (N–O distance –2.88 Å) between one of the ornithine side-chain nitrogen atoms and the D-phenylalanine carbonyl oxygen atom which had not been predicted. The other ornithine nitrogen atom cannot take part in such a hydrogen bond but has a close, potentially hydrogen-bonded, contact to a water molecule.

The crystal packing is apparently stabilised by many hydrogen-bonding interactions. For each gramicidin molecule there is one half-molecule of urea (occupying a site on a crystallographic 2-fold axis) and eight molecules of water (six in general positions, two on 2-fold axes and one disordered between two sites approximately 1 Å apart). All these small molecules are within hydrogen-bonding distance, either of the gramicidin molecule or of each other. In addition to the possible intermolecular hydrogen bonding involving the small molecules, there is a hydrogen bond between atoms in the backbone of two gramicidin molecules related by a 2-fold axis (N–O distance –2.73 Å). All the polar atoms in the gramicidin molecule are involved in short, probably hydrogen-bonding, contacts of one of the types mentioned above, as are all the solvent molecules.

We are continuing our efforts to obtain more accurate data for the structure.

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Thermoluminescence dating of sediment baked by lava flows of the Chaîne des Puys

AN earlier attempt¹ to use thermoluminescence (TL) for determining the age of Quaternary lava flows, and in particular of the magnetically reversed² Laschamp and Olby flows of the Chaîne des Puys in the French Massif Central, was frustrated by anomalous fading of the TL. This anomalous fading seems to be exhibited by most of the TL-sensitive minerals in lava and is probably due to wave mechanical leakage of electrons out of traps to nearby defects^{3,4}. It has not been observed in quartz¹ but only minimal quantities of this mineral are present in the basaltic flows of the Chaîne des Puys. Although the TL phototransfer technique is a promising⁵ way of using traps that are immune to anomalous fading, it has not yet proved possible to obtain TL dates directly from volcanic lava (although an empirical technique has achieved relative dating in one particular application⁶). We report here the TL dates for the baking by lava of clay and sediment, thereby avoiding the malign TL characteristics of the lava itself. TL dating of baked clay in the form of archaeological pottery is well established⁷ and the procedures developed in that application were followed here. Some of the dates obtained are of particular interest because of the likely association² of one of the lava flows concerned, that at Royat, with the magnetically reversed flows mentioned above. An average TL age for the latter of $33,000 \pm 4,000$ yr has been obtained by Valladas *et al.*^{8,9}; this is based on quartz extracted from a granitic inclusion found in the Laschamp flow and an underlying quartz pebble heated by the Olby flow.

Table 1 lists the results obtained from two exposures of baked sediment that had been heated by the Royat flow; this flow originated in the region of the Petit Puy de Dôme and moved down the valley of La Tirectaine through Fontanas and Royat to Chamalières (see ref. 10). The upper of these two exposures (samples *j1*, *j3.1*, *j3.2*, *j11* and *j42*—pointed out to M.J.A. by A. Roche in September 1976) lies in Royat on the North side of the Avenue de la Vallée facing the entrance to the car park (on the west side of which is La Grotte des Laveuses). The sediment at this location was plentiful and consisted of well consolidated red baked clay firmly adhering to lava at one boundary. Palaeomagnetic measurements by Barbetti and Flude (personal communication) on two orientated samples of the baked clay collected at the same time as those for TL gave directions within

Fig. 1 Plateau test; the ratio of natural TL to natural plus artificial TL as a function of glow-curve temperature, for *a*, reddened layer (*j1*); and *b*, unreddened layer (above *j1*). Other unreddened layers show similar behaviour. The artificial dose was 9 krad for *a* and 17 krad for *b*.

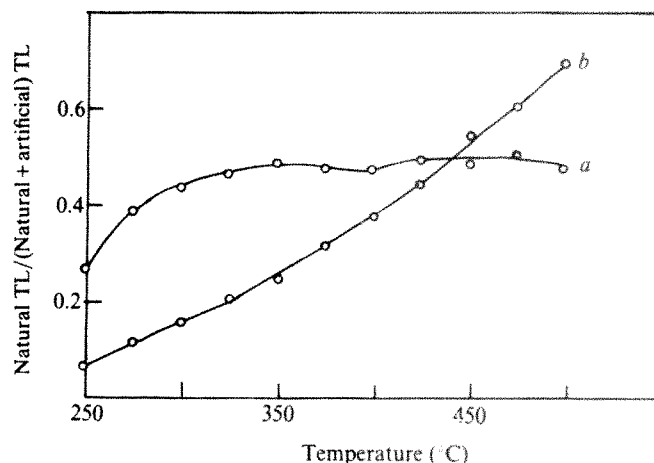


Table 1 Royat locations (45°45' N, 3°03' E)

Sample	Ancient dose (krad)	Water content (% of dry weight)	Dose-rates (rad yr ⁻¹)			TL age (yr × 10 ⁻³)
			Effective α	β	γ + cosmic	
Upper location						
j1	9.0	24	0.125	0.139	0.11	24 ± 2.7
j3.1	11.2	20	0.085	0.132	0.11	34 ± 3.6
j3.2	9.9	20	0.137	0.132	0.11	26 ± 2.7
j11	8.9	24	0.156	0.157	0.11	21 ± 1.8
j42	11.2	26	0.109	0.126	0.11	32 ± 3.2
Lower location						
j32	14.3	27	0.198	0.182	0.12	29 ± 2.8
j34	14.0	23	0.161	0.186	0.12	30 ± 2.6

Mean age (weighted) = 25.8 × 10³ yr; standard error = 1.7 × 10³ yr; predicted error = 2.2 × 10³ yr (random plus systematic).

3° of the rather abnormal result found^{11,12} for lava samples collected from the same location (declination 62° W, inclination +70°, α₉₅ = 3.5°; location 24 in ref. 12). Similar ancient directions for this flow both in respect of lava and in respect of associated baked sediment were found¹³ some 75 yr ago by Brunhes and David!

The lower location (samples j32 and j34) was on the east boundary of the car park itself, ~5 m below the upper location and 75 m nearer to the centre of Royat (see ref. 14). The sediment here is dark red and only ~1 cm thick; it is overlain by ~30 cm of ashy volcanic debris on top of which is the lava flow itself. The baked sediment here was poorly consolidated and although palaeomagnetic measurements by Barbetti and Flude (personal communication) showed some scatter, the sample mean directions were within 15° of the result previously obtained for the lava^{11,12} (declination 74° W and inclination +63°, α₉₅ = 3.5°; location CF 22 in ref. 12). A radiocarbon determination by Delibrias *et al.*¹⁵ for carbon extracted from the underlying soil at this locality gave an age of 11,070 ± 200 radiocarbon yr (Gif 2255) and on the basis that it is from a well protected palaeosol, this has been taken¹⁴ to be the maximum age for the lava. The palaeomagnetic results from the two locations indicate that they are stratigraphically equivalent.

The TL ages determined for these two locations, given in Table 1, were based on the fine-grain technique¹⁶; the error limits were evaluated as discussed elsewhere^{17,18}. The values for the combined γ + cosmic radiation dose-rates were obtained¹⁹ by burying small capsules of natural fluorite, for a year, in the sediment in a similar position with respect to the lava as had been occupied by the TL samples. Using our standard form of citation the average age derived from all seven samples is 25.8 × 10³ yr (±1.7 × 10³, ±2.2 × 10³, OxTL 132j). The two error limits quoted are^{17,18}, respectively, the standard error of the mean derived from the observed scatter of the individual ages, and the error predicted by taking into account random and systematic sources of uncertainty; the second limit is appropriate when comparing ages derived from other chronometric techniques.

There is a gross discordance between the TL age and the radiocarbon age mentioned above. This could arise in the radiocarbon determination if somewhat more than 20% of

the carbon in the sample was 'modern'. Although this is considered unlikely because of the well protected situation of the palaeosol and because of the pretreatment that was given to the sample to remove contaminating humic acid, this ¹⁴C age should be accepted with caution (G. Delibrias, personal communication, pointed out the desirability in these circumstances of evaluating the age of the humic acids). On the other hand, the TL age might be erroneously too old if there had been infiltration into the sample of unbaked sediment. This is inherently unlikely in the lower location because the overlying lava would have heated any material above the layer of burnt sediment, but error due to this factor is also considered to have been excluded by the following sample rejection procedure.

The presence of a significant amount of unheated material would be expected to cause a sample to fail the 'plateau test' that is routinely applied⁷ (see Fig. 1); this test demands that there is a substantial temperature region (350–450 °C in the present work) of the glow curve within which the ratio of ancient TL to artificially induced TL does not vary (by more than 10% in the present work). Measurements made, for each location, on the ashy material lying above the baked sediment showed a change in this ratio by a factor of about two between 350 and 450 °C; hence the 10% plateau criterion implies rejection of samples in which contamination exceeded 10%. This level might be serious if the equivalent dose carried by the contaminating material was vastly in excess of that carried by the baked sediment. However, the highest equivalent doses evaluated for overlying material were 7 krad at a glow-curve temperature of 350 °C and 18 krad at 450 °C. This corresponds to the overlying material having been heated at the time of the volcanic activity, as might be expected; that the equivalent dose at 450 °C is higher than that for the baked sediment correlates with a higher radioactivity, as observed, and the lower equivalent dose at 350 °C is consonant with the effects of the anomalous fading which, as mentioned previously, prevents TL dating of volcanic material itself. Similar plateau measurements were made on unreddened soil beneath the dated layer at the lower location; these indicated an equivalent dose of 16 krad at 350 °C and 40 krad at 450 °C.

The increase with depth of the equivalent dose (when evaluated at a glow-curve temperature of 350 °C) raises the question of whether the sediment was heated to a sufficiently high

Table 2 St Saturnin (45°36' N, 3°06' E)

Sample	Ancient dose (krad)	Water content (% of dry weight)	Dose-rates (rad yr ⁻¹)			TL age (yr × 10 ⁻³)
			Effective α	β	γ + cosmic	
i12 (quartz inclusions)	3.4	12	0	0.200	0.135	10.1 ± 1.3
i15 (quartz inclusions)	3.0	19	0	0.200	0.135	8.9 ± 1.8
i17 (fine grains)	3.1	24	0.156	0.185	0.137	6.5 ± 0.7

Mean age (weighted) = 8.1 × 10³ yr; standard error = 1.1 × 10³ yr; predicted error = 0.8 × 10³ yr (random plus systematic).

temperature for earlier TL to have been erased. Partial erasure is ruled out by the satisfactory plateau observed for the reddened layers—for partial erasure the degree of erasure at different glow-curve temperatures would vary strongly. Negligible erasure would have been highly unlikely so close to molten lava and can certainly be ruled out for sample *j1* which was actually adhering to a vertical face of lava which terminated the extent of the baked clay.

Accepting that the heating by the lava was sufficient for complete erasure, there is also the possibility that because of anomalous fading the age of 26×10^3 yr may be erroneously too young. Tests on artificially irradiated portions of all the samples listed in Table 1 showed that for a storage period of 6 months any decay was less than experimental uncertainty (3%). However, this does not rule out a significant effect over thousands of years and an attempt was made to utilize the quartz inclusion technique—as there was no evidence that quartz suffers from this malign phenomenon. Unfortunately, the yield of quartz was too small to allow the necessary comprehensive measurements for a reliable date but for sample *j1* the equivalent dose was estimated as 6–9 krad; this corresponds to an age of between 26×10^3 and 35×10^3 yr. Thus although one cannot rule out a small amount of anomalous fading in the fine grains it does not seem to have had a gross effect.

In conclusion, bearing in mind the suggestion² that because of its abnormal magnetic direction, the Royat flow may be associated with the magnetically reversed Laschamp and Olby flows, the TL results for the Royat flow are mutually reinforcing with those obtained by Valladas *et al.*^{8,9} for Laschamp and Olby. Note that the radiocarbon age for the first phase of the geomagnetic polarity excursion²⁰ detected in aboriginal hearths at Lake Mungo, Australia, is $\sim 30 \times 10^3$ yr (5,730-yr half-life), and that the thermoluminescence age²¹ for these hearths is $(33.5 \pm 4.3) \times 10^3$ yr.

We have also carried out TL measurements on clay baked by the lava flow at St Saturnin. The radiocarbon age for this flow, being based on wood carbonised by the lava, is much more secure than the palaeosol determination for the Royat flow. Hence it is of particular interest to ask whether in this case the TL dating is consistent with the radiocarbon determinations. The TL samples, of black baked clay, were collected from above the pathway below the chateau, close to the spot where carbonised wood for ^{14}C dating was obtained by A. Rudel. Unfortunately, the TL characteristics were not all satisfactory and in view of this, the agreement (see Table 2) with the ^{14}C age of $7,650 \pm 350$ radiocarbon yr (Sa90) obtained by Pelletier *et al.*²² is better than could be expected; using the 5,730-yr half-life, the ^{14}C age becomes 7,880 yr and eventual availability of bristle cone pine calibration may show the corresponding calendar age to be greater still. The relevant γ dose-rates were obtained, as for the Royat locations, by burial of small capsules of natural fluorite. Table 2 shows that the standard error is comparable with the predicted error; this reflects the unsatisfactory TL characteristics mentioned above and indicates incidentally that in this application the system for evaluation of predicted error has been insufficiently pessimistic.

Finally, for some samples (provided by G. Camus), measurements were undertaken to check whether or not the flows concerned belonged to the period of Quaternary volcanism. As capsule measurement was not feasible for these samples the γ dose-rate had to be estimated from radioactive analysis of the material presumed to surround the sample. The fine-grain age evaluated for a sample of grès (from the quarry behind 120 Ave du Mont Dore, Beaumont) that had been heated by the Coulée de Boissejour was $\sim 50 \times 10^3$ yr and measurements made on quartz inclusions from this sample were consistent with this. Various other materials from this quarry were tried (baked clay, grès feldspathic) but none had sufficiently satisfactory TL characteristics for a reliable TL age to be determined. Clay from Montpoly that had been baked by the Coulée de Beaumont gave a fine-grain age of 34×10^3 yr. For calcaires trempés from the Butte de Clermont the plateau test⁷

indicated that there had been strong fading in the 330°C peak—indicating either that the material had not been heated in the eruption or that, on the basis of the equivalent dose indicated by the 420°C peak, it had been heated more than half a million years ago.

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Changing He/Ar and N₂/Ar ratios of fault air may be earthquake precursors

THE geochemical method for the prediction of earthquakes depends on the ability to find the anomalous change of fluid phases of the ground, which are expelled by the stress at depth before earthquakes. Here the utility of ratios of He/Ar and N₂/Ar in fault airs is examined in this context

The radon emanation of groundwater may be a useful indication of the precursory effects of earthquakes¹. Helium may also be of interest, because unlike radon, helium does not decay but accumulates in the lithosphere. Use of only a single element for predicting earthquakes, however, is less reliable in some cases. A rare gas in the groundwater is lost through bubbling² and the concentration of the rare gas is lowered by other gases such as methane and carbon dioxide formed in the ground. Hence, it seems most appropriate to study the relative concentrations among gases and the ratios of He/Ar and N₂/Ar discussed here may prove to be the technically most useful parameters.

Helium and argon produced in the lithosphere are estimated from the abundances of radioactive elements (Table 1), and the ratio of helium to argon found to remain constant for the past several hundred million years irrespective of rock types. The ratio in the lithospheric air (~ 10) is 10^5 times higher than that in the atmospheric air (5.7×10^{-4}). The ratio in the atmospheric air becomes double the usual ratio because of the contamination of

Table 1 Abundances of U, Th and K in rocks and He/Ar ratios in the lithospheric air derived from the radioactive elements

	U	Th	K	He/Ar*	
	(p.p.m.)	(p.p.m.)	(%)	Myr	100 Myr
Basalt	0.83†	5†	0.58‡	10.8	10.8
Granite	3.96†	13.5†	2.50§	8.82	8.82
Shale	3.7	12	2.24¶	8.95	8.95

* The year represents the accumulation interval. The decay constants used for the calculation are as follows: $\lambda_U = 1.537 \times 10^{-10} \text{ yr}^{-1}$, $\lambda_{Th} = 4.99 \times 10^{-11} \text{ yr}^{-1}$, $\lambda_K = \lambda_{EC} + \lambda_{\beta} = 5.29 \times 10^{-10} \text{ yr}^{-1}$, $\lambda_{EC} = 5.7 \times 10^{-11} \text{ yr}^{-1}$. The abundance of ^{40}K in potassium is assumed to be 0.0119%.

† General average⁹.

‡ Average in Quaternary Japanese basalts¹⁰.

§ Average in Japanese granite¹¹.

|| General average¹².

¶ Average in Japanese shales¹³.

0.005 vol % of the lithospheric air. Table 2 shows some examples of natural gas composition in Japan and USA. The concentrations of helium and argon are often lower than those in the atmospheric air, whereas the He/Ar ratios are much higher. This indicates that the lithospheric air permeates through the natural gases. The lithospheric air released by the formation of cracks in rocks, therefore, can be easily detected using this ratio.

The ratio N_2/Ar is also expected to be a useful parameter of lithospheric air, because the ratio in natural gases and volcanic gases is generally higher than that of the atmospheric air (85.21) (refs 3–6). The origin of nitrogen in the lithospheric air has been explained in terms of (1) the release of nitrogen by the bacterial decomposition of nitrogen-bearing compounds, (2) the release of nitrogen by the inorganic chemical breakdown of organic materials, and (3) the release of inorganic nitrogen from igneous (and metamorphic) rocks^{4,5}.

The ratios He/Ar and N_2/Ar are measured by gas chromatography without vacuum processes, temperature and pressure corrections and other complicated treatments. This technique is simple enough for amateur observers to make the appropriate measurements.

Since 1976 I have continuously measured the variations of He/Ar and N_2/Ar ratios of gas bubbles found in the water issuing from a fault zone almost 2 m wide which runs in chert and shale of the Palaeozoic. The fault crosses the precinct of Inuyama Crustal Movement Observatory of Nagoya University which is ~20 km north of Nagoya.

The gas bubbles are constantly gathered in a bottle set upside down in the water, then transferred into a glass flask with high vacuum stopcocks. Some of the gas sample (2 ml) is injected into the gas chromatograph, tank oxygen is used as a carrier gas. Helium, argon and nitrogen are separated through a molecular sieve 5 Å column at a room temperature, and the three peaks of these gases emerge on a recorder chart within 10 min. The relative areas of peaks correspond to the relative volumes of

gases in the sample and the mutual ratios among these gases are determined by the atmospheric air as a reference standard in the same conditions. Error (2σ) in the determination is ~10% for He/Ar ratios and ~2% for N_2/Ar ratios. If the gases were each measured by a different method, the error would inevitably be increased. The single procedure provides sufficient precision for analysing the ratios.

The successive measurement indicated that a high He/Ar ratio (0.014) was accompanied by the earthquake of 26 July 1976 (magnitude: 4.1; epicentral distance: 67 km), but the sampling interval (once or twice a month) was too long to decide whether the high ratio occurred before or after the shock⁷. The sampling interval was therefore shortened after March 1977 (once in two or three days).

Some correlation between occurring of earthquake and change of gas quality was observed, but the variation of the observed ratios has no relationship to the records of tiltmeter, extensometer and precipitation obtained in the observatory. The ratios of He/Ar and N_2/Ar fluctuated considerably: for the next 10 measurements, the moving average for data smoothing was calculated after March 1977. The results are plotted in Fig. 1, which shows that a few peaks appeared. The four vertical

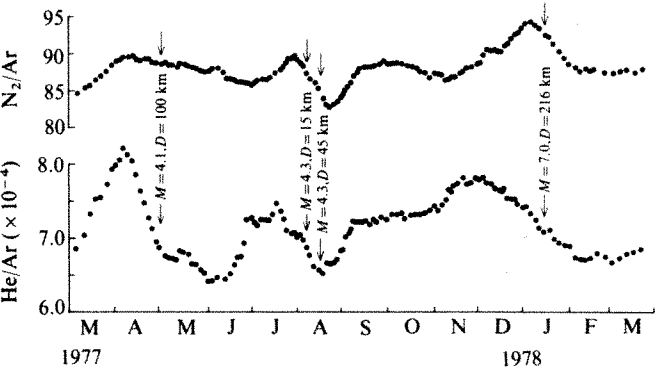


Fig. 1 The temporal variations of He/Ar and N_2/Ar ratios in gas bubbles from the fault zone. The original data sequence was smoothed by 10-term moving averages after the usual process in statistics¹⁴. The vertical arrow indicates the earthquakes with the magnitude ≥ 4 in the vicinity of Nagoya. *M* represents magnitude and *D*, epicentral distance. See also Table 3.

arrows show the earthquakes (magnitude ≥ 4) which occurred in the vicinity of Nagoya during the observation. The three events of the four were felt in Nagoya (Table 3). Figure 1 shows explicitly that the peaks appear before the earthquakes. This correlation found can hardly be chance and suggests that the change of gas composition is closely related to the occurrence of an earthquake. The epicentral distance of the earthquake on 14 January 1978 amounts to 216 km, which greatly exceeds the size

Table 2 Examples of natural gas compositions

No.	CH ₄	C ₂ H ₆	N ₂	CO ₂	He	Ar	He/Ar	N ₂ /Ar
			(%)					
<i>a</i>								
KR-5-2	97.1	2.11	0.48	0.17	0.00055	0.0060	0.092	80
KR-9-2	96.9	1.87	0.43	0.59	0.00066	0.0039	0.17	110
<i>b</i>								
2	95.43	2.37	1.57	0.09	0.0041	0.0052	0.79	302
3	95.12	3.96	0.86	0.06	0.0037	0.0067	0.55	128

a, Nishinagaoka, Niigata, Japan^{3,4}.
b, Rio Vista, Sacramento, California⁵.

Table 3 The earthquakes (magnitude ≥ 4) occurred in the vicinity of Nagoya during the measurements of Fig. 1

Date	Magnitude	Distance from the epicentre (km)	Intensity in Nagoya*
30 April 1977	4.1	100	0
6 August 1977	4.3	15	III
15 August 1977	4.3	45	I
14 January 1978	7.0	216	III

* Intensity expressed on the scale of the Japanese Meteorological Agency

of the dilatant volume generally accepted. According to the observation in China⁸, however, anomalous radon contents of groundwater were recorded at distances less than 110 km from the shock, for events in the magnitude range 4.3–5.0; for the larger quake of magnitude 7.9 in the Pohai Gulf, the wells showing radon anomalies were located at distances of 250–300 km. These observations suggest that the changes in gas composition reflect sensitively stresses which precede earthquakes and such changes could be precursors of earthquakes.

Several aspects arise from Fig. 1, in terms of earthquake prediction. The duration of the high ratio is longer for one large earthquake (14 January 1978, magnitude 7.0) than those for the small ones (magnitude ≈ 4). Another aspect is that the peaks of He/Ar precede those of N₂/Ar for all events. This might be attributed to the difference in diffusion velocity of He and N₂ through capillary cracks formed by stress before earthquake. The molecular weight of N₂ is 28 and of He is 4; the ratio of diffusion velocities is $\sqrt{28/4} = 2.65$ and He migrates more rapidly by diffusion than does N₂. It is also noticeable that the time lag of the N₂/Ar peak for the large earthquake is longer than for the others. Thus, by estimating both the time lag of N₂/Ar peak and the duration of high ratios, one could predict the occurrence time and magnitude of the approaching earthquake. Furthermore, observation in many locations may provide a basis for predicting where the earthquake will occur.

The earthquake of 14 January 1978 caused a disaster in the Izu Peninsula. Many observatories and seismology and geodesy instruments such as tiltmeter, extensometer and seismograph have been installed around the peninsula. These instruments did not record the premonitory phenomena for the earthquake except for a radon meter and a Sacks–Evertson strain meter. On the other hand, the method described here clearly records the precursory changes in gas compositions at the epicentral distance of 216 km. It seems therefore that this method is a promising practical means of predicting earthquakes.

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Seawater/basalt ratio effects on the chemistry and mineralogy of spilites from the ocean floor

PILLOW basalts metamorphosed to the greenschist facies have been frequently dredged from mid-ocean ridges^{1–3}. These rocks usually exhibit a distinct mineralogical differentiation in which the pillow rim or margin is replaced by chlorite or, less commonly, epidote. Calcic plagioclase and olivine within the pillow interior or core are replaced to varying degrees by albite and chlorite, respectively². Actinolitic amphibole, pyrite, quartz, sphene, and small amounts of nontronite and saponite are the other alteration phases identified in these rocks^{1–3}. Corresponding to this mineralogical differentiation is a chemical differentiation characterised, in general, by enrichment of MgO and H₂O in the pillow rim relative to the core; the core is in turn enriched in Na₂O, CaO and SiO₂. Cann⁴, in his study of the metamorphosed pillow basalts dredged from the Carlsberg Ridge, Indian Ocean, refers to the Mg-enriched, Ca-depleted, chloritised rind of the pillows as hyalospilites, and the Na-enriched cores as orthospilites. Metabasalts similar in chemistry and mineralogy to those dredged from mid-ocean ridges have also been found in ophiolites, obducted portions of oceanic crust now exposed on continents^{4,5}. The oxygen- and strontium-isotopic composition of these basalts^{6,7}, and the oxygen-isotopic composition of secondary minerals separated from dredged basalts⁸, suggest that alteration resulted from interaction with seawater at temperatures from about 200 to 350 °C. Passage of seawater through a sequence of pillow basalts would most probably occur along fractures and cracks between individual pillow tubes. In this regard, Spooner *et al.*^{6,7} from studies of the extent of hydration, oxidation and oxygen- and strontium-isotopic composition of metamorphosed pillow basalts at Troodos, Cyprus, have concluded that the flow of seawater through basalt pillow piles occurred primarily along fractures and pillow boundaries, whereas grain-boundary diffusion was the principal mechanism of mass transfer accompanying chemical reaction within the solid rock. Because of this variation in permeability, pillow rims would be exposed to a much greater volume of seawater relative to the crystalline cores; consequently, alteration of the rim would proceed at a higher effective water/rock ratio⁹. We propose here that the water/rock mass ratio during seawater–basalt interaction affects the stability of secondary minerals and therefore accounts for

Table 1 Chemical composition of basalt glasses

Component	Fresh	Altered*	Altered†
SiO ₂	50.10	46.44	43.36
Al ₂ O ₃	14.80	14.18	13.74
Fe ₂ O _{3(t)}	12.60	12.30	11.50
MgO	7.00	8.59	15.24
CaO	11.10	7.84	1.64
Na ₂ O	2.57	2.59	0.89
K ₂ O	0.14	0.04	0.06
TiO ₂	1.78	1.69	—
H ₂ O ⁺	0.50	6.40	11.00
Total	100.59	100.07	97.43

—, Not analysed.

* Altered basalt glass after interaction with seawater at 350 °C, water/rock ratio 10.

† Altered basalt glass after interaction with seawater at 300 °C, water/rock ratio 62.



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the mineralogical and chemical diversity between the core and rim of metamorphosed pillow basalts. To demonstrate this, we report the change in mineralogy and chemistry of basalt glass reacted with seawater at 300 and 350 °C, 500 bar at water/rock mass ratios of 62 and 10, respectively. The experimental system and analytical procedures are discussed in refs 10, 11.

Interaction of seawater with basalt glass at 350 °C, 500 bar, and water/rock mass ratio of 10 results in the formation of a mineral assemblage containing albite, saponite, mixed-layered chlorite-saponite, actinolitic amphibole and anhydrite, as well as minor prehnite, quartz and pyrite. In general, this alteration assemblage is similar to that in the cores of metamorphosed basalts. Furthermore, because of chemical exchange with seawater during this experiment, the basalt glass gained Na₂O, MgO and H₂O and lost CaO, SiO₂ and K₂O, while total iron and Al₂O₃ were conserved (Table 1). These directions of chemical

Table 2 Gains (+) and losses (–) of chemical components resulting from formation of orthospilite or hyalospilite from fresh Indian Ocean Ridge basalt², and during experimental spilitisation of basalt glass

Component	Orthospilite	Basalt glass*	Hyalospilite	Basalt glass†
SiO ₂	+7.02	–1.63	–7.77	–3.39
Al ₂ O ₃	—	—	—	—
Fe ₂ O _{3(t)}	+0.19	–0.10	+10.78	+0.46
MgO	+1.15	+1.97	+4.98	+9.46
CaO	–2.16	–2.92	–8.93	–9.32
Na ₂ O	+1.88	+0.13	–1.14	–1.61
K ₂ O	–0.06	–0.10	–0.08	–0.08
H ₂ O ⁺	+2.83	+6.18	+7.76	+11.38

Al₂O₃ has been conserved.

* Spilitisation at seawater/basalt mass ratio 10.

† Spilitisation at seawater/basalt mass ratio 62.

exchange, and for some species even the magnitude, are strikingly similar to those inferred during the transformation of tholeiitic basalt to orthospilite (compare columns 1 and 2 of Table 2). The one exception is SiO₂, which is enriched in the orthospilite samples described by Cann². During experimental 'spilitisation', SiO₂ is lost from basalt because fresh seawater contains very little SiO₂ and the tendency is for the solution to become saturated with quartz. Whether a rock in the natural setting becomes enriched or depleted in SiO₂ depends in general on whether the solutions which pass through it are supersaturated, as in the case of a cooling solution, or undersaturated with quartz. The enrichment observed by Cann may thus not be typical for orthospilites as a whole.

Further evaluation of the data (Table 2) reveals that, although Na is enriched in basalt during this experiment, the degree of enrichment is minor compared with that of the typical orthospilite. The formation of albite from basalt glass or calcic plagioclase depends critically on the Na/Ca ratio of the aqueous phase. If this ratio is large and remains large during seawater-basalt interaction, the basalt would generate potentially greater amounts of a sodic phase such as albite at the expense of calcic phases such as epidote or prehnite. Although seawater has an initially high Na/Ca ratio, reaction with basalt within a closed system results in a significant decrease in this ratio and in the tendency to form albite. For example, in the present experiment the Na/Ca molal ratio of the solution decreased from 45 to 13, partly by loss of Na but mainly by gain of Ca (M.J.M. and W.E.S., unpublished data). In nature, however, this build-up of Ca in the intergranular 'pore' solution within the crystalline core would be free to diffuse into zones having a lower Ca concentration and a higher Na/Ca ratio, such as the relatively less altered, more freely circulating seawater infiltrating interpillow channels (Fig. 1). Concomitantly, Na in this fluid could then

diffuse into the core and become fixed as albite; this process would account for the Na-rich chemistry of orthospilites. It is important to note, however, that if circulation of seawater within the interpillow channels is impeded for some reason, a decrease in Na/Ca ratio can be expected. This would alter the diffusion gradients of Ca and Na and ultimately result in the formation of calcic silicates as vein fillings. Phenomena of this sort may help to explain the 'epidote-rich' greenstones reported by Humphris and Thompson³, in which epidote typically occurs in veins.

Seawater-basalt interaction at 300 °C, 500 bar, and a water/rock ratio of 62 represents a seawater-dominated system¹², in which most of the CaO, Na₂O and K₂O as well as some of the SiO₂ are leached from the basalt (Table 1). At the same time, MgO and H₂O are added to the altered basalt, which was converted entirely to a mixed layered chlorite-smectite. The only other phases which formed were anhydrite and very minor haematite.

The exchange of components between seawater and basalt during alteration at a high water/rock ratio agrees well with the chemical gains and losses inferred from the chemistry of the chloritised rind (hyalospilite) of pillow basalts dredged from the Carlsberg Ridge (Table 2). Furthermore, interaction at a high water/rock ratio produces a solution appreciably enriched in Fe, Mn, Cu, Zn and SiO₂. The precipitation of these components, either in channels within the pillow pile or on the seafloor could explain the widespread jasper and ferromanganous sediment commonly associated with spilitised basalt.

The mineralogical and chemical heterogeneity commonly observed in metamorphosed seafloor basalts can, therefore, be explained on the basis of interaction with seawater at various water/rock ratios. Experimental seawater-basalt interaction at

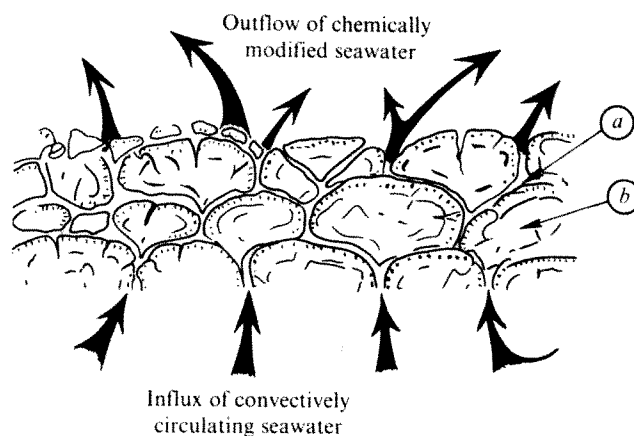


Fig. 1 Schematic illustration depicting circulation of seawater through pillow basalt pile undergoing hydrothermal alteration: a, primary zone of Mg-metasomatism (high water/rock ratio); b, zone of alteration characterised by diffusional metasomatism (low water/rock ratio).

a low effective water/rock ratio produces an alteration assemblage characterised by albite, actinolite, prehnite, chlorite-smectite, quartz, pyrite and anhydrite. At a high water/rock ratio (62), in contrast, the basalt is strongly leached and replaced by chlorite-smectite. These results apply directly to the natural system, in which, because of the inherent permeability of pillow basalt piles, the pillow core has limited direct access to seawater and alters at a low water/rock ratio, whereas the glassy selvage of the pillow exterior apparently comes into contact with much greater volumes of seawater and consequently alters at a high water/rock ratio.

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Adaptive value, entropy and survivorship curves

NATURAL survivorship curves fall into three main types^{1,2}. Type I, or rectangular distribution, describes the situation in which all individuals attain the maximum physiological longevity of the species. Here the maximum age at death and the mean life expectancy coincide. The Type II life table describes a mortality which is independent of age; that is, no single age group is favoured at the time of dying. The Type III table is characterised by a high mortality early in life and a life expectancy which increases with the age of survivors. The three types of curve are illustrated in Fig. 1. The general shape of the survivorship curve is fixed for each species, however, the convexity of the curve is highly sensitive to environmental conditions and the genetic constitution of the population. This fact suggests that the general shape of the life table may provide information about the genetic variability in the population, the range of environmental factors that impinge on the population or the incidence of random events in the lifetimes of different individuals³. This note characterises in terms of two new demographic parameters the relative effect genetic and environmental factors play in the evolution of the life table. The analysis we give revolves around two demographic variables, the entropy of a population and the adaptive value. Entropy H measures the variability of the mortality distribution. The measure of variability also describes the convexity of the life table. The adaptive value ψ is a measure of the correlation between the variability of the mortality distribution and the environmental variability. Thus the adaptive value reduces to the entropy when the environment is constant.

We give two distinct characterisations of life-history curves in terms of optimisation arguments. In the first characterisation, we assume that the survivorship curves are completely determined by the genetic constitution. The life tables are derived by maximising H subject to constraints which are given a genetic interpretation. In the second description, we assume that the environmental effects are the only significant influence. The three life tables are derived in terms of maximising ψ subject to constraints on the environmental parameters. We

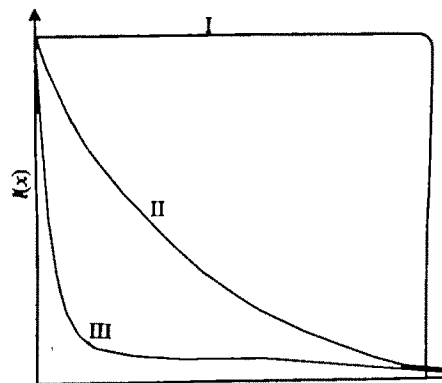


Fig. 1 The theoretically possible distinct types of life table. For Type I, $H = \log e^0$; for Type II, $H = 1 + \log e^0$ and $l(x) = \exp -(x/e^0)$; for Type III, $H = \frac{1}{2} + \log e^0$ and $l(x) = \exp -(\pi/4e^{0.2})x^2$.

also discuss the corresponding biological mechanisms which generate the life tables. Note that the genetic and environmental constraints which are imposed in the optimisation arguments correspond to initial conditions in the mechanistic description. These biological mechanisms can be mathematically described in terms of a birth and death process. This mathematical model together with a precise description of the correspondence between constraints and initial conditions is discussed elsewhere⁴.

Let $l(x)$ be the probability that an individual born at age zero survives to age x , and let $m(x)$ be the age-specific fecundity. The net reproductive rate R_0 is given by

$$R_0 = \int_0^\infty l(x)m(x) dx$$

The expectation of life of an individual aged x is

$$e^0 = \int_0^\infty l(x) dx$$

The entropy of the population⁴ is given by

$$H = - \int_0^\infty q(x) \log q(x) dx$$

where $q(x) = l(x)m(x)/R_0$ is the probability density function of the age of reproducing individuals. The expression H is a measure of the variability of the contribution of the different age-classes to the stable age-distribution. It also measures the convexity of the net-maternity function $V(x) = l(x)m(x)$. Fixing the fecundity, say $m(x) = 1$, we obtain

$$H = - \int_0^\infty \frac{l(x)}{e^0} \log \frac{l(x)}{e^0} dx \\ = - \frac{\int_0^\infty l(x) \log l(x) dx}{\int_0^\infty l(x) dx} + \log e^0$$

Normalising, we obtain

$$H^* = \frac{\int_0^\infty l(x) \log l(x) dx}{\int_0^\infty l(x) dx}$$

The expressions H and H^* measure the convexity of the survivorship curve. For the Type I life table, $H^* = 0$ and $H = \log e^0$. For the Type II life table, $H^* = 1$, and $H = 1 + \log e^0$.

An interesting application of our entropy parameter to the study of male-female mortality patterns is given in ref. 5.

The notion of an adaptive value of a population⁶ generalises the entropy concept to incorporate the effects of environmental forces on the life-history distribution. We refer to ref. 6 for a precise mathematical definition. In this note we give an intuitive account. We consider the population as a stationary stochastic process whose phase space, the set of all life histories is subject to perturbations by an environmental process. There

are thus two stochastic processes at work, the process described by the population and the process described by the distorting effects of the environmental action. We can therefore identify two entropies, H , the entropy of the population in the case when no environmental forces interfere, and $\hat{H}$, the conditional entropy, that is, the entropy of the perturbed process given that the unperturbed mortality distribution is known. The adaptive value ψ is defined to be

$$\psi = H + \hat{H}.$$

This function measures the correlation between the variability of the mortality distribution and environmental variability. Thus, in a constant environment, ψ is precisely the entropy H of the population.

We now describe the various distributions that maximise H subject to various constraints. We suggest biological interpretations of these constraints by specifying mechanisms which implement these adaptive strategies.

(1) The distribution that maximises H subject to the constraint

$$\int_0^{\omega} l(x) dx = 1$$

where ω is the maximum life expectancy, yields the Type I life table.

The mechanisms implementing this strategy can be described in terms of a mortality which is the end result of senescence or wearing out of the individual, the senescent process going on at the same time and at the same rate in each individual.

(2) The distribution that maximises H subject to the constraint on the mean of the distribution $l(x)$ is given by

$$l(x) = \exp\left(\frac{-x}{e^0}\right)$$

This yields the Type II life table. This curve describes great interindividual variation in the age at death. The variation can arise exclusively from interindividual genetic variability. In the Type I curve, there is no genetic variability, hence the senescent process is the same in each individual. This implies that the life expectancy and the maximum longevity of the species coincide. For the Type II curve, there is genetic variability, hence senescence is less uniform. The degree of genetic variability of the population is measured by the constraint on the mean of the mortality distribution.

(3) The distribution that maximises H subject to a constraint on the variance is given by

$$l(x) = \exp\left[\frac{-\pi}{4e^{0.2}}\right] x^2$$

Of the three curves described, Type III represents the greatest individual variability in the time of dying. The constraint on the variance is a measure of the genetic variability in the population. The senescent process in this case is much less uniform than that which generates the Type II life table.

It is important to note that these distributions can also be derived as a result of maximising the adaptive value ψ , subject to environmental constraints. Thus an environmental agent that is lethal to individuals irrespective of their genetic constitution and that kills off all individuals at some prescribed age ω will yield a life table whose variability is maximally correlated with the environmental action, that is, a Type I curve.

An environmental agent that acts randomly on the individuals of different ages and is indifferent to the ages of the individuals will yield a Type II curve. Finally, an environmental agent that is age-specific and selectively removes younger individuals will yield a Type III table.

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Egocentric orientation is influenced by trained voluntary cyclorotary eye movements

A CYCLOROTARY eye movement is a motor response of the eye made around the visual axis. Counter-rolling of the eye, for example, occurs during lateral head tilt^{1–5}; conjugate rotary nystagmus can be induced by a large rotating field^{6–9}; and disjunctive cyclotorsions can occur during ordinary convergence^{10,11}. Because none of these torsional eye movements can be produced as an isolated voluntary response, eye torsion has always been classified as an involuntary response, a reflex. Using a visual-feedback procedure, however, we have trained humans to make conjugate voluntary cyclotorsional eye movements up to 30 degrees in magnitude¹². We have also demonstrated that these large torsional movements are not visually induced and can be made in the absence of any visual stimulus. Accompanying the training and performance of these eye movements were a number of striking illusions related to one's own sense of body orientation. Because these newly trained eye movements are unprecedented, it is of interest to characterise accompanying illusions in detail, comparing them with other illusions of self rotation induced through vestibular¹³ and visual^{14,15} inputs. In this paper we compare the effects of trained cyclorotary eye movements with head and whole body tilts, showing a quantitatively similar change in egocentric orientation for each type of tilt. As such, our findings suggest the possibility of shared mechanisms affecting the stability of one's internal frame of reference, both for eye and body movements.

The exact method of training and testing of voluntary torsion using visual biofeedback has been detailed elsewhere¹². A subject was seated in a dark room with head movements fixed by a full mouth bite plate. A vertical $11 \times 0.25^\circ$ flash was presented monocularly to generate a vertical afterimage. This afterimage was then imaged in space next to a line of light of equal colour, brightness and size (real line stimulus). The subject was instructed to keep this afterimage line matched parallel to the real line only by cyclorotating the eyes. With practice, the subject gradually acquired the ability to rotate the eye(s) to match greater and greater inclinations of the real line. These cyclorotations were trained at the rate of about $0.8^\circ \text{ h}^{-1} \text{ d}^{-1}$ for 35 d (Fig. 1a). Using similar methods for 5–10 h of additional training, subjects were trained to make torsional slow pursuit (Fig. 1b) and torsional saccades (Fig. 1c). Individual frame analysis of 35-mm slides or 16-mm motion pictures filmed simultaneously were used to objectify the cyclorotary eye movements. Measurements from photographs used radial iris markings or limbal-scleral blood vessel junctions in relation to a visible and stable reference marker. Accuracy of measurements was ± 10 min.

During initial training, but not during final experimentation, subjects often felt that their heads and bodies were 'rolling laterally' and sometimes experienced associated visual illusions. Accompanying these changes in egocentric orientation were intermittent sensations of stomach nausea, headache and general body fatigue. The most dramatic of these illusions occurred as a distinct 'hallucinatory barrage' at least twice to each subject during initial training. Associated with eyelid tremor, the nearly vertical and parallel lines would appear to move independently, becoming almost horizontal and also appearing curved or wiggly. Accompanying these peculiar visual illusions were very powerful sensations of body flotation

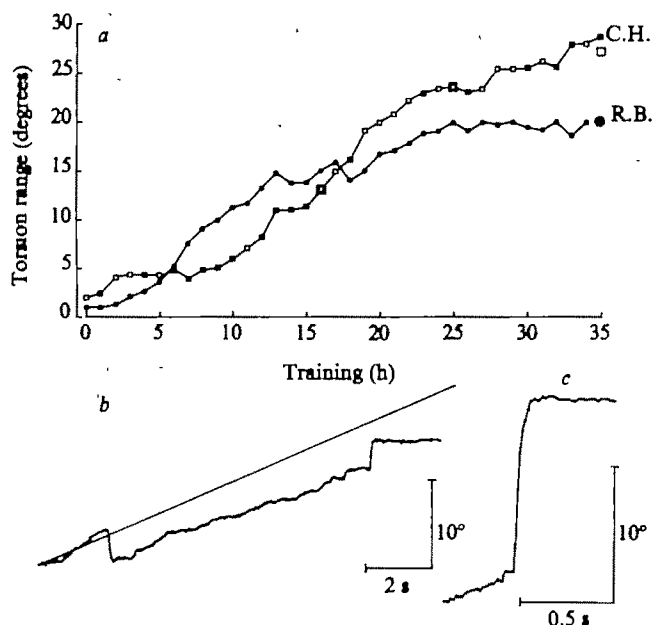


Fig. 1 *a*, Training of voluntary torsion. Maximum torsional range measured psychophysically (small symbols) or photographically (large symbols) plotted against hours of training. *b*, Voluntary cycloverlational slow pursuit. Solid line represents a tracing moving at the correct velocity of 1.6° s^{-1} . Photographed at 24 frames s^{-1} . *c*, Time course of a voluntary torsional saccade. Photographed at 64 frames s^{-1} .

and/or rapid downward falling in the direction of the voluntary cyclotorsion. Although self-induced, these sensations show similarities to those experienced by pilots in conditions in which conflicting visual and orientational information can occur¹⁶. It should be noted that these gross and episodic disorientations occurred only during initial training.

In this report, we restrict our quantitative observations to a more constant and milder sensory illusion of body tilt, persisting even after a long period of training. To examine this shift in egocentric orientation and to make comparisons with other situations involving vestibular and neck proprioceptive input, we have devised a control and three test situations. Two subjects felt a rod pivoted about its centre so as to rotate in the frontal plane and adjusted it to the apparent vertical in the dark under each of the following conditions (Fig. 2, top): (1) the real line stimulus was viewed in otherwise dark surroundings at one of several angles of tilt (S); (2) voluntary eye torsion was used to match an otherwise vertical afterimage to specific tilts of the real line (E); (3) a head tilt was used to match the orientation of the real line to that of the afterimage (H); and (4) head and body were both tilted by means of a rotating chair in complete darkness (B).

All tilts and rotations were presented in 3° increments over a range of $\pm 9.0^\circ$. In condition H, the reflexive counter-torsion of the eye was less than 1° (average 0.6°) for even the largest 9° tilts; consequently, actual tilts of the head are slightly underestimated. Before each trial, the experimenter randomly displaced the rotatable rod to ensure that subjects could not memorise hand or rod positions.

The results are graphed in Fig. 2 (bottom). Condition S, as a control, demonstrated that the presence of the inclined real stimulus line had essentially no influence on the subjects' sense of vertical, changing it by only 3% of the real line's tilt. Such a line has also been shown to induce essentially no torsional movements of the eyes¹². Therefore, it can be concluded that the presence of the inclined real line in conditions E and H is of little consequence.

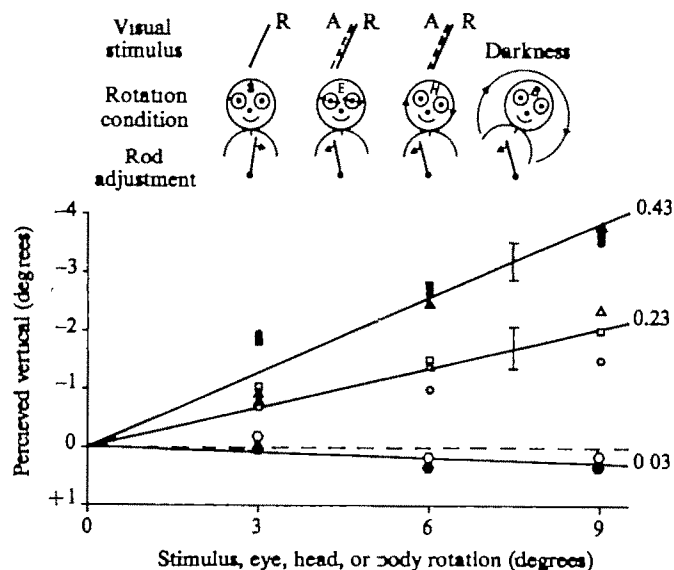
Voluntary torsion (condition E), however, caused marked shifts in the position of apparent vertical as did the two other

conditions (H and B). All adjustments were overestimated in the opposite direction to eye, head or body tilts and by an amount which was virtually identical in all three conditions (Fig. 2). It should also be noted that the results for conditions H and B confirm previous reports^{17,18}. Because the results are so similar in the three conditions, we averaged the data and plotted a single regression line for each subject, arriving at a slope of 0.23 for subject C.H. and 0.43 for subject R.B. If 1.0 is defined as a gain factor whereby a perfect vertical rod adjustment could be made, a gain factor for the subjects' sense of vertical or egocentric orientation can be determined by adding 1.0 to each slope. Thus, C.H. and R.B. overestimated their turning by an average factor of 1.23 and 1.43, respectively.

These experiments suggest that for each subject, there is a constant overcompensation signal determining egocentric orientation, regardless of whether the eye is voluntarily rotated (voluntary cyclotorsion), the otoliths and neck proprioceptors are stimulated (head tilt condition), or the otoliths are stimulated without significant neck proprioception stimulation (whole body tilt). In this case of voluntary torsion, as well as for other examples of the perceptual effects of eye torsion^{17,19}, the compensatory signal could arise either as an outflow, originating from a torsional command centre, or as an inflow, originating from eye muscle receptors. The results do not indicate the source of the compensatory signal.

What is surprising is that the motor act of voluntary torsion, which is independent of vestibular or visual stimulation, can, by itself, induce quantitatively identical sensations to actual head and body rotations. This similarity suggests that there might be a common pathway for each effect. Electrophysiological

Fig. 2 A laterally rotatable rod was adjusted to perceived vertical in the dark by subjects in different conditions of rotation over a range of $\pm 9^\circ$ (top). The visual stimulus for each condition is shown: R, real line; A, afterimage line. Both R and A are set to vertical when eyes are at rest and straight ahead. Rotation conditions are defined as follows: S, real line stimulus rotation only (control); E, voluntary eye torsion defined by an afterimage match to tilts of the real line; H, head tilt defined by an afterimage line match to tilts of the real line; B, body and head tilt in complete darkness. Graph shows the change in perceived vertical as a function of rotation. Rod settings for conditions E, H and B were in the opposite direction to any direction of physical rotation (abscissa) by a factor of about one-third (average slopes of 0.23 and 0.43 for subjects C.H. and R.B.). Control condition (S) showed essentially no effect (average slope = 0.03). $n = 40$ for each data point. Error bars on regression lines indicate average s.e. mean for the combined data of C.H. (bottom line) and R.B. (top line), based on 468 df. R.B.: $\bullet$, S; Δ , E; $\blacksquare$, H; $\circ$, B; C.H.: $\circ$, S; Δ , E; $\square$, H; $\circ$, B.



recordings from the vestibular nerve, for example, show that moving visual scenes produce signals that are similar to a motion stimulus to the semicircular canals²⁰⁻²³, a result which could be the physiological basis for the recent finding that egocentric orientation can be altered by rotating visual fields¹⁴. Perhaps a similar sharing of circuitry exists with respect to the newly trained response of voluntary eye torsion.

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Possible role of excretory/secretory products in evasion of host defences by *Fasciola hepatica*

RATS develop a marked resistance to reinfection with *Fasciola hepatica* after a single immunising infection^{1,2}. This resistance is apparent within 2 weeks of initial infection^{3,4} but diminishes in long-standing infections⁴. Resistance does not depend on the presence of the initial infection at the time of challenge², and operates against intraperitoneally implanted metacercariae, juvenile or adult flukes³ as well as against orally administered metacercariae. Implantation (subcutaneous or intraperitoneal) with adult flukes does not stimulate marked resistance to challenge^{3,5}, and these implanted flukes may persist for many months in the cysts which form around them in the rat. Resistance may be transferred by cells⁶ or, provided that it is given at the time of challenge, by immune serum⁷; serum administered 6-8 d after infection is not effective⁷. However, the flukes of the immunising infection persist in the rat in an apparently normal healthy condition^{1,2} and clearly evade the mechanism which controls challenge infections. We report here experiments which show that *F. hepatica* produces substances which are toxic to its host's lymphocytes and that these substances may protect the parasite from its host's immune defences.

Liver fluke excretory/secretory products (ESP) were obtained by incubating adult flukes, collected from sheep or rats, in dry, stoppered plastic tubes for 1-2 h at 37 °C. During this time the flukes expelled the major part of their caecal contents. This was separated by centrifugation and stored at -20 °C until required for use. ESP were tested for toxicity to rat splenic lymphoid cells using the method of van Furth and van Zwet⁸ and

Table 1 Effect of ESP on immunoadhesion of peritoneal cells to *F. hepatica*

Replicate		% Flukes with adherent cells		
		Control	Immune control	ESP
1	1	0	91.7	11.1
	2	0	73.8	16.7
	3	14.3	61.5	4.3
	4	10	76.0	8.3
	5	0	76.2	14.3
Mean		4.9	75.8	10.9

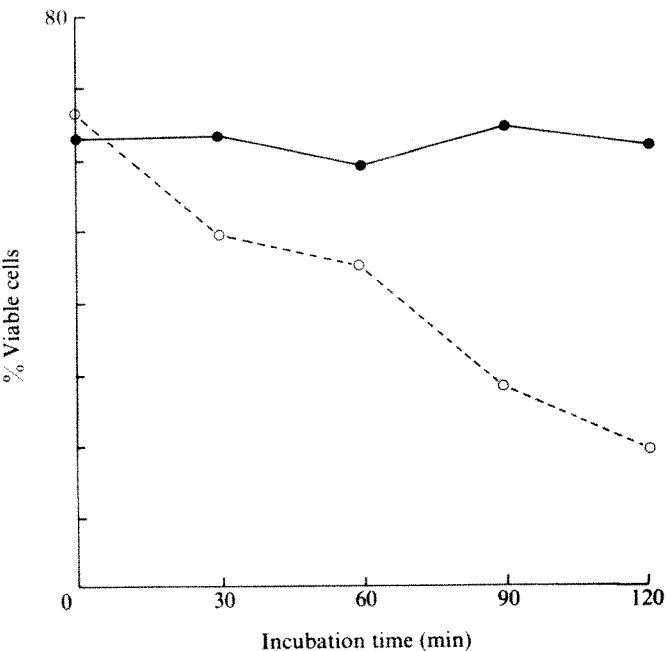
0.1-ml volumes of juvenile *F. hepatica* (~2,000 per ml) and immune rat peritoneal cells (6×10^6 per ml) in tissue culture medium 199 were incubated with 0.1 ml fresh normal rat serum+0.1 ml medium 199 (control), 0.1 ml fresh immune rat serum+0.1 ml medium 199 (immune control) or with 0.1 ml fresh immune rat serum+0.1 ml ESP (ESP). The % of flukes with adherent cells was noted after 90 min incubation at 37 °C. Mean of control compared with mean of immune control, $P < 0.001$; mean of immune control compared with mean of ESP, $P < 0.001$.

the results are shown in Fig. 1. Viability of control cells remained at the fairly constant level of about 60% throughout the 2-h incubation period, whereas cells incubated in the presence of ESP showed a decrease in viability from 66% preincubation to 19% after 2 h.

The relevance of this finding to the evasion of host defences by *F. hepatica* was demonstrated using an *in vitro* immunocytoadhesion system. Juvenile *F. hepatica* were obtained by the *in vitro* excystation of metacercariae. When incubated *in vitro* with fresh serum and peritoneal cells from *F. hepatica*-resistant rats, these juvenile flukes become heavily coated with cells which include many eosinophils (Fig. 2). No cellular adhesion is seen if serum from uninfected rats is used.

The effect of including *F. hepatica* ESP in this *in vitro* system is seen in Table 1. Incubation in normal rat serum gave a mean of 4.9% flukes with cells attached, whereas incubation in immune serum gave 75.8% flukes with attached cells. Inclusion of *F. hepatica* ESP as well as immune serum reduced the numbers of flukes with adherent cells to 10.9%. This reduction was statistically highly significant (Student's *t*-test).

Fig. 1 Toxicity of ESP to lymphoid cells *in vitro*. 0.2-ml volumes of normal rat splenic lymphoid cells (15×10^6 per ml) in tissue culture medium 199 were incubated with 0.2 ml medium 199 (solid line) or 0.2 ml ESP (broken line). At 30-min intervals equal volumes of cell suspension and 0.2% Trypan blue were mixed and the % of viable cells determined.



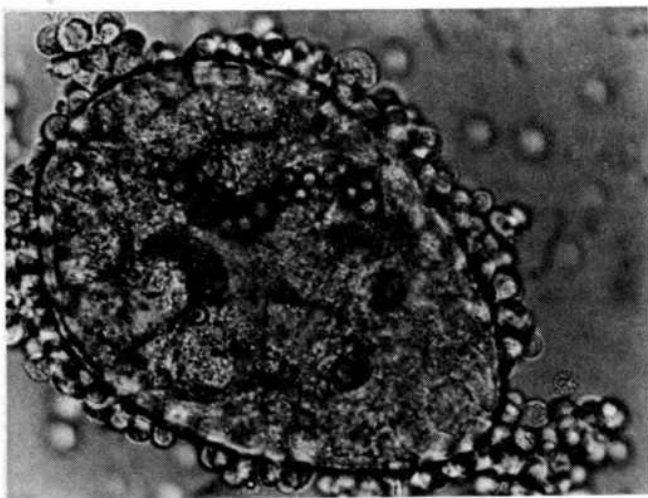


Fig. 2. Immune adhesion of peritoneal cells to *F. hepatica*. 0.1-ml volumes of juvenile *F. hepatica* (~2,000 per ml) and immune rat peritoneal cells (6×10^6 per ml) in tissue culture medium 199 were incubated at 37°C for 15 min with 0.1 ml fresh immune rat serum. Wet preparation 400× magnification.

Cellular attachment is likely to be a first step in the immune killing of *F. hepatica* and the above results clearly show that *F. hepatica* ESP prevent this attachment in the *in vitro* situation. It seems highly probable that ESP also have a similar role *in vivo*, where they are maintained in intimate contact with the fluke in the bile duct or in the fluke's migratory tract in the liver.

It is thus proposed that the actively feeding liver fluke protects itself from host immune defences by producing substances which are toxic to its host's lymphocytes and other immunocompetent cells. This hypothesis is consistent with certain facts known about *F. hepatica* and its relationship with the rat. For example, very young flukes not producing effective quantities of ESP would be expected to be the immunogenic stage of infection, and older, actively feeding flukes would be nonimmunogenic. The early development of resistance after initial infection, the waning resistance in long-standing infections and the lack of resistance after implantation with adult flukes are thus explained. The importance of timing of passive serum transfer is also explained; transfer at the time of challenge, before flukes are producing ESP will be effective, but transfer later, when ESP are being produced in effective quantities, will not confer protection.

Further details of *in vitro* and *in vivo* immune adhesion of cells, particularly eosinophils, to *F. hepatica* will be published elsewhere, and electron microscopic studies of the immune killing of *F. hepatica in vitro* and *in vivo* are in progress.

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Membrane noise and conductance increase during single spermatozoon-egg interactions

ALTHOUGH one of the earliest events of fertilisation of the sea urchin egg is a depolarisation and decrease in resistance of the egg plasma membrane¹⁻³, it is not known exactly what change occurs immediately after the interaction with a single spermatozoon. We report here that in *Paracentrotus lividus* this change is a depolarisation step of 1-2 mV, occurring 13 s before the main electrical change. The step is accompanied by an increase in cell noise and a decrease in resistance. In other biological membranes, such an observation has been interpreted as the introduction of ionic channels into the membrane^{4,5}. From this analogy we calculate that the single channels introduced into the egg plasma membrane during fertilisation have a conductance greater than 33 pS.

At sperm densities of 10^7 ml⁻¹ or greater we observed a fertilisation action potential similar to that already reported by Steinhardt *et al.*¹ (their Fig. 3) and Ito and Yoshioka³ (their Figs 2 and 3). In their recordings the depolarisation was composed of two phases. The initial phase has been called the shoulder of the action potential³. Closer observation of our records shows that the shoulder is composed of discrete events.

Progressively reducing the sperm density changed the configuration of the shoulder. For example, at a sperm density of 10^6 ml⁻¹, a shoulder was composed of three or four discrete steps, whereas at densities below 10^5 ml⁻¹ it was composed only of a single step. At very low densities, such as those used in Fig. 1a, it was not uncommon to wait several minutes for a reaction to occur. Both single steps and shoulders lasted for 13 s at 22°C.

The single step is the minimum detectable event during the shoulder phase of the fertilisation action potential. Eggs displaying the minimum event cleave normally. As sea urchins cleave abnormally if more than one sperm enters the egg, we deduce that a step corresponds to the successful interaction of the fertilising spermatozoon. Eggs which display shoulders cleave abnormally and are polyspermic.

Rothschild and Swann^{6,7} have shown for *Psammochinus miliaris* that at sperm densities of 10^5 ml⁻¹, the number of sperm-egg collisions will be 0.16 s⁻¹. The probability for a successful collision was measured as 0.12, so that on average, the successful encounter rate should be once every 50 s. This supports our interpretation that a step corresponds to one successful encounter.

A typical step and fertilisation action potential is shown in Fig. 1a. Steps were always accompanied by an increase in voltage noise. Lowering the temperature prolonged the step. At 22°C the average duration was 10.9 ± 1.8 s ($n = 12$), and at 12°C the average duration was 25.0 ± 4.3 s ($n = 3$). Similarly the rise time of the fertilisation action potential decreased from 1.13 ± 0.54 mV s⁻¹ to 0.27 ± 0.09 mV s⁻¹.

Figure 1b shows a step during an experiment carried out at 5°C in which the duration was 79 s. The longer step allowed a reasonable time for spectral analysis of the noise increase, and for an estimate of the change in variance before and after the step. This analysis is shown in Fig. 2. The voltage noise during the step increased primarily in the low frequencies. At rest, membrane resistance may range from 4 to 18 KΩ cm² (egg diameter 80 μm); during a step the resistance was always reduced by one-third to one-half.

To obtain an estimate of the elementary conductance change contributing to a step, we calculated the ratio of the change in noise variance to the change in potential. This ratio for Fig. 1b was 10 μV, which may be taken as the size of the elementary event contributing to the noise. Taking the specific resistance of

the egg during the step at its mean value of $6 \text{ K}\Omega \text{ cm}^2$ ($30 \text{ M}\Omega$), the current associated with this elementary event was 0.33 pA . To calculate the conductance through which this current passes, an estimate of the equilibrium potential of the step is required.

Progressively offsetting the resting potential towards zero, using a second intracellular electrode, resulted in a gradual decrease in the size of the step. At -3 mV , a step was difficult to observe and the noise increase was minimal; however the fertilisation reaction still occurred. This experiment suggested an equilibrium potential near zero. It was not possible to invert the step as fertilisation was suppressed when the egg was held positive, as reported elsewhere⁸.

Using the data from Fig. 1b, with a resting potential of 10 mV , the elementary conductance was calculated to be 33 pS . Repeating the calculation for three other examples gave the values 16 , 41 and 92 pS . In none of these cases was the resistance measured simultaneously with the noise. These values are large compared with the conductance of ion-specific channels, for example, Na and K channels in nerve membranes; however, they are similar to nonspecific drug-induced channels^{4,5}.

In the absence of Ca^{2+} a step did not occur; if calcium was added a normal step was observed and development ensued. The step seems to be a general phenomenon in sea urchins, as we have also seen it in *Psammechinus* and *Arbacia*.

What is the relationship between the electrical events constituting a step and the morphological events which occur during fertilisation? Possibilities include labile attachment of the spermatozoon to the vitelline membrane, strong attachment either to the vitelline membrane or egg plasma membrane, or fusion of the two gametes. Also, the acrosomal reaction is

Fig. 1 *a*, Intracellular recording from a mature egg of *Paracentrotus lividus* during fertilisation. Sperm density was 10^3 ml^{-1} . Egg jelly was removed by acid seawater ($\text{pH } 5.0$). The fertilisation action potential is the transient change in membrane potential shown in the latter half of the upper trace. The fertilisation potential is preceded by a step. The upper trace was d.c.-coupled whereas the lower trace, a.c. coupled from 1 Hz , demonstrates the noise increase during the step. (The noise trace saturates during the fast rise of the fertilisation action potential, and is omitted.) Vertical bar represents 12 mV (upper), 1 mV (lower); horizontal bar represents 5 s and also indicates the zero potential level. Temperature was 25°C . *b*, A step at 5°C . Vertical bar represents 6 mV (upper), 1 mV (lower); horizontal bar represents 5 s and indicates zero potential level.

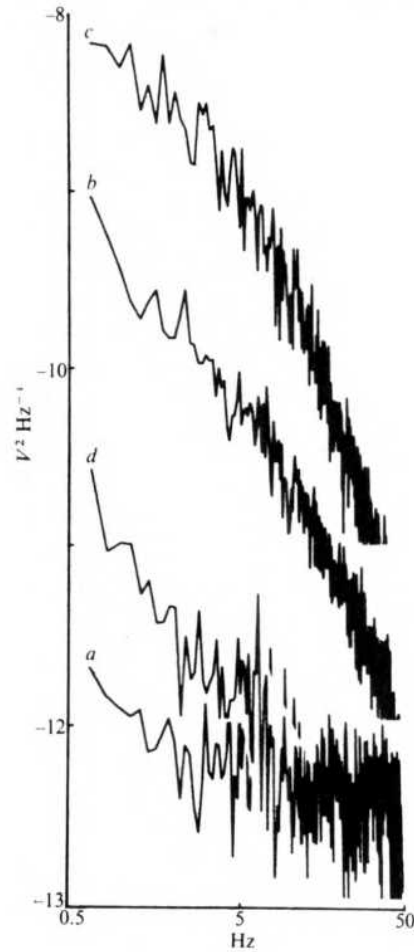
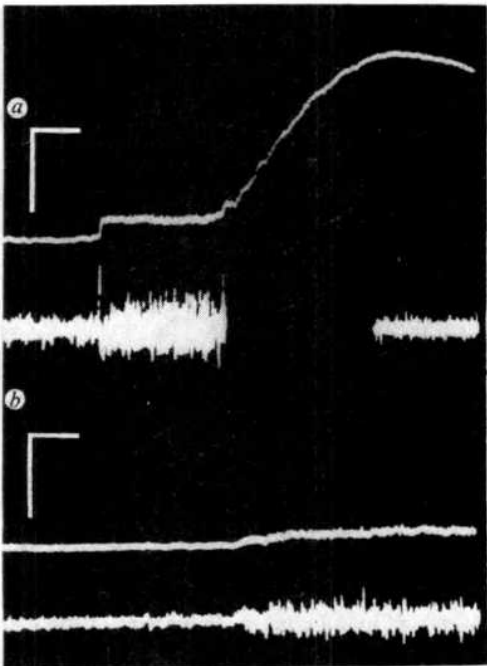


Fig. 2 Power spectral density of voltage noise from the experiment shown in Fig. 1b. *a* microelectrode noise before the experiment; *b*, cell noise before the step; *c*, during the step; *d*, microelectrode noise after pulling out of the egg. About 40 s of data were used to estimate *b* and *c*; only half this amount for *a* and *d*. The microelectrode noise although increased after the experiment was well below the cell noise during the step. The spectra are limited by a.c. coupling at 1 Hz , and the time constant of the membrane.

generally considered as a prerequisite to sperm entry⁹. Aketa and Ohta¹⁰ have shown in *Pseudocentrotus* that the acrosomal reaction occurs following attachment to the vitelline membrane.

Arbacia spermatozoa made labile attachments to *Paracentrotus* eggs, but such attachments were not accompanied by an electrical change. However, at $\text{pH } 9.4$ a normal step and fertilisation action potential occurred qualitatively similar to that recorded for homologous fertilisation of either species. High pH alone did not activate the eggs; however, *Arbacia* sperm exposed to high pH underwent the acrosomal reaction as reported elsewhere¹¹. We deduce from this evidence that the step is subsequent to the acrosomal reaction. This interpretation would also explain the absence of the step in Ca^{2+} free seawater because the acrosomal reaction is Ca^{2+} dependent¹¹.

We suggest that a factor is released from the acrosomal vesicle which causes directly or indirectly the introduction of conductance channels into the egg plasma membrane. Introduction of many conductance channels into a membrane would result in a depolarisation and a noise increase and a decrease in resistance^{4,5}. Although our results do not rule out cell fusion as giving rise to the step, the conductance increase cannot be explained by the introduction of a simple hole into the egg plasma membrane.

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Enzymatic methylation of phosphatidylethanolamine increases erythrocyte membrane fluidity

PHOSPHOLIPIDS are a major component of biomembranes and provide the fluid matrix for protein movement and organisation. Membrane fluidity has been closely related to the biological and biochemical processes such as transport of chemicals, cell fusion, and protein rotation and diffusion^{1,2}. Genetic, nutritional and temperature manipulation have been used to alter the fluidity of the cell membrane^{3,4}, but physiological and biochemical events that regulate membrane fluidity are still unknown. We report here that the enzymatic methylation of phosphatidylethanolamine in erythrocyte membranes induces a marked increase of the bulk membrane fluidity.

An examination of the role of phospholipid methylation in the regulation of membrane fluidity was prompted by the find that two methyltransferases are involved in the conversion of phosphatidylethanolamine to phosphatidylcholine⁵. These enzymes were found to be asymmetrically distributed in the erythrocyte membrane. The first enzyme that converts phosphatidylethanolamine to phosphatidyl-N-monomethylethanolamine faces the cytoplasmic side, whereas the second enzyme that adds two additional methyl groups from S-adenosyl-L-methionine to form phosphatidylcholine is located on the external surface of the erythrocyte membrane⁶. The substrate for the first methyltransferase, phosphatidylethanolamine, resides mainly on the cytoplasmic side and the product of the second methyltransferase, phosphatidylcholine, is present largely on the external surface of the membrane^{6,7}. Thus, translocation of phospholipids from the inside to the outside of the membrane is facilitated by a stepwise methylation of phospholipids. The enzymatic methylation of phosphatidylethanolamine to phosphatidylcholine takes place within the membrane, Mg²⁺ playing a critical part⁶. To examine the effect of such phospholipid rearrangement across the membrane on the lipid fluidity, the following experiments were carried out.

Fresh samples of blood were obtained from ether-anaesthetised male Sprague-Dawley rats (150 g) by cardiac puncture using heparin as anticoagulant. The erythrocytes, washed with 0.9% NaCl solution, were haemolysed with 40 volumes 5 mM Tris-phosphate buffer, pH 8.0, containing 1 mM MgCl₂ (ref. 8). The ghosts thus obtained were suspended into 10 volumes 50 mM Tris-glycylglycine buffer, pH 8.0, containing 5 mM MgCl₂. After centrifugation at 4 °C, 0.3 ml of packed ghosts were suspended in 0.7 ml of the same buffer, containing various

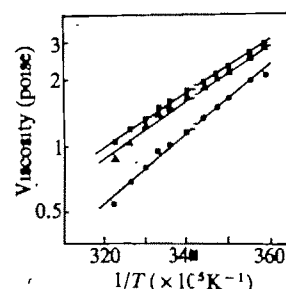


Fig. 1 Effects of incubating erythrocyte ghosts with S-adenosyl-L-methionine and S-adenosyl-L-homocysteine on membrane microviscosity. Erythrocyte cell ghosts were preincubated at 4 °C in 5 mM MgCl₂ and 50 mM Tris-glycylglycine buffer, pH 8.0, containing a 50 μM S-adenosyl-L-methionine (●); b, 50 μM S-adenosyl-L-methionine and 2 mM S-adenosyl-L-homocysteine (▲); c, control (□). The ghosts were then, resealed and labelled with DPH at 37 °C for 1 h. The amount of methyl group incorporated into lipids was 9 pmol per mg protein and 0.5 pmol/mg protein in the absence and presence of S-adenosyl-L-homocysteine, respectively. The calculation of the microviscosity at 25 °C was made according to the method of Hare *et al.*¹⁰.

concentrations of S-adenosyl-L-methionine and/or S-adenosyl-L-homocysteine, and incubated overnight (approximately 16 h) at 4 °C. Before use, the ghosts were washed with 10 volumes of a solution containing 150 mM NaCl, 10 mM Tris-HCl buffer, pH 8.0, and 5 mM MgCl₂ (solution A). The fluorescent probe, 1,6-diphenyl-1,3,5-hexatriene (DPH) was used for the measurement of microviscosity by the method of Shinitzky and Barenholz⁹. Fluorescence labelling was carried out by incubating the red blood cell ghosts in the solution A containing 1 μM DPH at 37 °C for 1 h. Labelled cells were washed and suspended in solution A at a concentration of 0.1-0.2 mg cell protein per ml.

The microviscosity of the erythrocyte membrane was found to be a function of temperature but no phase transition was apparent over a temperature range of 6 to 36 °C (Fig. 1). S-adenosyl-L-methionine was introduced inside the ghosts by preincubation at 4 °C. When the resealed ghosts were labelled with DPH at 37 °C for 1 h, the methylation of phospholipids as determined by procedures described previously⁵ took place simultaneously, but no significant methylation was observed at 4 °C. When S-adenosyl-L-methionine was present inside the ghosts, the microviscosity markedly decreased (Fig. 1). This reduction was almost completely abolished when S-adenosyl-L-homocysteine was present inside the cells together with S-adenosyl-L-methionine. S-adenosyl-L-homocysteine inhibited the methylation of phosphatidylethanolamine and itself did not affect the membrane microviscosity. These results indicate that

Table 1 Correlation between microviscosity changes and amount of phosphatidyl-N-monomethylethanolamine formed

SAM (μM)	[³ H]-methyl incorporation (pmol per mg protein)			Microviscosity (P)
	PME	PDE	PC	
0	—	—	—	1.62
4	1.15	<0.01	<0.01	1.32
12	1.80	0.1	0.1	1.18
25	1.90	0.50	0.40	1.08
50	1.95	1.20	1.50	1.06
100	1.97	2.40	3.60	1.09

SAM, S-adenosyl-L-methionine; PME, phosphatidyl-N-monomethylethanolamine; PDE, phosphatidyl-N,N-dimethylethanolamine; PC, phosphatidylcholine. Varying amounts of S-adenosyl-methyl-³H-L-methionine were introduced into rat erythrocyte ghosts and incubated as described in Fig. 1. The methylated phospholipids formed enzymatically were quantified after separation by thin layer chromatography with the solvent system, n-propyl alcohol/propionic acid/chloroform/water (3/2/2/1 v/v/v).

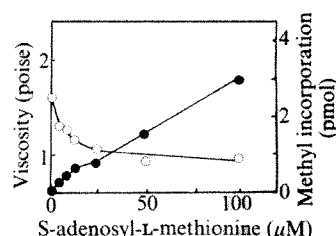


Fig. 2 Effects of various concentrations of S-adenosyl-L-methionine on microviscosity. Erythrocyte blood cell ghosts were preincubated at 4 °C with various concentrations of S-adenosyl-methyl-³H-L-methionine and then labelled with DPH at 37 °C to measure microviscosity. A part of the mixture equivalent to 0.2 mg protein was examined for ³H-methyl incorporation into the lipid fractions after extraction with 3 ml of chloroform/methanol/1 M HCl (6/3/1)^{5,6}.

thle methylation of phospholipids alters the membrane fluidity.

Previous work has shown that the enzymatic methylation of phosphatidylethanolamine has a low K_m for S-adenosyl-L-methionine (1.4 μ M), whereas the methylation of phosphatidyl-N-monomethylethanolamine to phosphatidylcholine had a high K_m for the methyl donor (0.1 mM)⁵. To determine whether synthesis of phosphatidyl-N-monomethylethanolamine and/or phosphatidylcholine was responsible for the changes in fluidity, varying concentrations of S-adenosyl-L-methionine were introduced into erythrocyte ghosts. Figure 2 shows the effects of increasing concentration of S-adenosyl-L-methionine on membrane viscosity. The concentration of S-adenosyl-L-methionine required for half-maximal reduction of the microviscosity was approximately 5 μ M. A plot of the amount of methyl group incorporated against S-adenosyl-L-methionine concentration shows an inflection point at about 25 μ M, indicating the presence of two enzymes in the methylation of phospholipids (Fig. 2). The identity of the methylated phospholipids at various concentrations of S-adenosyl-L-methionine were measured by introducing the methyl-³H labelled compound into the erythrocyte ghosts. When the methylated products were isolated and quantified using thin layer chromatography⁵, monomethylated phosphatidylethanolamine was found to form predominantly at concentrations of S-adenosyl-L-methionine below 25 μ M (Table 1). In these conditions, the membrane microviscosity reached nearly maximum. At concentrations above 25 μ M, phosphatidylcholine were increasingly formed (Table 1). These results indicate that the erythrocyte membrane fluidity changes are caused mainly by the enzymatic methylation of phosphatidylethanolamine to phosphatidyl-N-monomethylethanolamine. In contrast to phosphatidylethanolamine and phosphatidylcholine, we have found that phosphatidyl-N-monomethylethanolamine is deeply embedded in the erythrocyte membrane⁶. It is not susceptible to the digestion by phospholipase C from either the inside or outside erythrocyte ghosts. The accumulation of phosphatidyl-N-monomethylethanolamine within the lipid bilayer could account in greater part for changes of membrane fluidity.

Alterations in membrane fluidity due to phospholipid methylation should produce pleiotropic changes in membrane function. Preliminary results already indicate that binding of a catecholamine agonist with the β -adrenoceptor stimulates an increase of phospholipid methylation in reticulocytes and facilitates the lateral mobility and coupling of the receptor with adenylate cyclase (F. H., W. J. Strittmatter and J. A., unpublished).

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Recognition and genetic control of helper determinants for cell surface antigen Thy-1

THE hapten-carrier effect in immune responses demonstrates that one antigenic determinant can strongly modulate the antibody response to a second determinant if the two exist in physical association. This phenomenon, in more general form termed associative recognition¹, has been analysed with soluble antigens and reveals collaboration between carrier-directed, helper T cells and hapten-directed, antibody-producing B cells. In contrast to soluble antigens, antibody responses to surface antigens of nucleated cells are poorly understood and although collaboration among T and B cells is indicated in several studies^{2–4} the antigenic sites recognised by these T cells and the extent of associative recognition among the different antigenic determinants on cell membranes is not known. Thus, it is not clear whether the determinants recognised by T and B cells are controlled by a single gene and are joined covalently or if the two determinants are controlled by independent genes and are only loosely associated or even unassociated on the cell surface. This knowledge would help determine the character and extent of molecular interactions on the surface of nucleated cells as well as principles of immunogenicity of antigens expressed on cell membranes. This report extends previous findings^{1,5} and describes a new experimental approach to study, in primary responses the role of alloantigens in the immunogenicity of Thy-1 through the use of closely related donor-recipient strains and an assay for antibody plaque-forming cells (PFC). The results are consistent with the notion that in primary immune responses Thy-1 is 'helpless' but that in appropriate donor strains, help may be supplied by other alloantigens controlled elsewhere in the donor genome.

To examine the contribution of other alloantigens to the immunogenicity of Thy-1 we immunised AKR/J (Thy-1.1) mice with thymus cells from AKR/Cum or CBA (both Thy-1.2). Since AKR/Cum is closely related to AKR/J^{6,7} differences between the immunogenicity of CBA and AKR/Cum cells could be attributed to alloantigens of CBA donors absent in AKR/Cum cells. CBA thymus cells induced a large anti-Thy-1.2 antibody response in AKR/J mice which was measured as numbers of direct PFC per spleen (Table 1). This response peaked at 7 d while AKR/Cum thymus cells induced few or no PFC at any time. The response is fully specific for Thy-1.1 determined as before⁸ and considered further below. Two possible reasons for this difference in immunogenicity apart from the contributions of other donor alloantigens were considered; first that AKR/Cum thymus cells express much less Thy-1.2 than CBA cells, was excluded in serological tests which showed them to be indistinguishable in the ability to absorb anti-Thy-1.2 antibody, and second that the Thy-1.2 antigen of AKR/Cum is suppressive, is unlikely since mixtures of CBA and AKR/Cum cells are immunogenic (data not shown) and since in a more rigorous control the antigen on (CBA $\times$ AKR/Cum) F_1 hybrid cells is highly immunogenic (Table 1).

The augmentative effect of alloantigens was further explored in other strain combinations and with cells having the reciprocal Thy-1 alloantigen. (CBA $\times$ AKR/Cum) F_1 mice were

immunised with thymus cells from strain AKR, (AKR × AKR/Cum) F_1 or (AKR × CBA) F_1 all of which produced few or no PFC in the recipients (Table 2). However, thymus cells from (AKR × B10.BR) F_1 mice were highly immunogenic. Each of the four donors can be shown to be immunogenic for Thy-1.1 in the appropriate recipients, however in the present experiment only the (AKR × B10.BR) F_1 cells contain numerous alloantigens to which the recipients are not genetically tolerant. Thus a Thy-1 incompatibility is insufficient to produce strong PFC responses and other alloantigens seem to augment Thy-1 immunogenicity. This result moreover demonstrates that in F_1 hybrid antigenic cells, chromosomes from one parent can control determinants which help the response to another antigen controlled by independent chromosomes from the other parent. These findings have been confirmed in a second strain pair (A/Thy-1.1 and A/Thy-1.2) in which large numbers of PFC were induced by A/Thy-1.1 cells in B10.A mice (H-2) compatible but otherwise allogeneic) but very few in congenic A/Thy-1.2 recipients.

A nonimmunological process may be proposed to explain the augmentative effects of an allogeneic haplotype, however this is unlikely. For example, it would be otherwise difficult to explain how the CBA donor haplotype augments the anti-Thy-1.2 response in AKR recipients to (CBA × AKR/Cum) F_1 (Table 1) but the same haplotype fails to augment the anti-Thy-1.1 response to (CBA × AKR) F_1 in (CBA × AKR/Cum) F_1 recipients (Table 2). The immunological basis of the effect is supported by evidence of thymus-dependency described below. Moreover, in a concurrent but more complex study of secondary PFC responses to Thy-1 in which reciprocal specificity controls are possible we have found the recognition of the non-Thy-1 helper determinants to be immunologically specific (E. A. Clark and P.L., unpublished).

In classical hapten-carrier systems it is held that these two antigenic components must be firmly associated for the haptenic determinant to be immunogenic; mixtures of molecules are not active⁹. To test if the Thy-1 determinant and the putative helper determinants need to be physically associated, (CBA × AKR/Cum) F_1 mice were immunised with two cell preparations. The first inoculum comprised (AKR × B10.BR) F_1 thymus cells which express both the B10.BR and the Thy-1.1 alloantigens on

Table 1 Weak primary response of AKR to Thy-1.2 alloantigen on closely related donor cells

Dose	Day	Donor strain/PFC per spleen (s.e.m.)		
		CBA(Thy-1.2)	AKR/Cum(Thy-1.2)	
4×10^7	3	39 (26)	0 (0)	
4×10^7	5	519 (237)	21 (19)	
4×10^7	7	2241 (252)	15 (8)	
4×10^7	9	270 (52)	90 (60)	
4×10^7	11	39 (10)	51 (18)	
		CBA	AKR/Cum	(CBA × AKR/Cum) F_1
8×10^7	7	3429 (861)	0 (0)	2,100 (542)
4×10^7	7	2,241 (252)	15 (9)	1,221 (258)
2×10^7	7	1269 (510)	30 (30)	909 (441)

Homogeneous pools of AKR mice (all mice were bred by ICRF) were arranged in groups of four and inoculated on the appropriate days i.v. with 4×10^7 donor thymus cells which has been washed three times in Eagle's minimal essential medium (MEM). Spleens were removed from all the recipients at one time and were individually dispersed with a loose fitting homogeniser in 10 ml of MEM supplemented with 10% heat-treated (56 °C, 30 min) newborn calf serum (CA₁₀). The spleen cells were centrifuged (800 r.p.m. for 10 min) and resuspended in 1.5 ml of MEM. Thymus cells were obtained from CBA mice aged 3 weeks, washed three times as above and resuspended at 10% v/v in MEM-CA₁₀. 50 μ l samples of the spleen cell suspensions were mixed with 20 μ l of the target thymus cells in 2 ml capacity plastic tubes at 37 °C and 180 μ l of 0.95% Agarose in MEM-CA₁₀ at 45 °C were added. The cell suspension was applied directly to a 35 mm plastic Petri dish, incubated, treated with complement and stained as described⁸.

Table 2 Immunogenicity of Thy-1 is helped by other donor alloantigens

Thymus donor	Thy-1 phenotype	Anti-Thy-1.1 PFC per spleen (s.e.m.)
AKR	Thy-1.1/1.1	75 (19)
(AKR × AKR/Cum) F_1	Thy-1.1/1.2	48 (7)
(AKR × CBA) F_1	Thy-1.1/1.2	0 (0)
(AKR × B10.BR) F_1	Thy-1.1/1.2	4,328 (680)

(CBA × AKR/Cum) F_1 mice which were age and sex matched were arranged in groups of five recipients each and were injected i.v. with 4×10^7 of the indicated cell suspension. PFC were determined after 7 d. No difference in immunogenicity of male and female donors was detectable in several controlled experiments and subsequently both donors were used.

each cell and the second was a mixture of B10.BR and AKR cells where the helper alloantigens and the Thy-1.1 determinants are on separate cells. It is evident from the results in Table 3 that the helper alloantigens must be expressed on the cells which carry the Thy-1 determinant for augmentation to result. Mixtures of cells fail to show any premium effect. This indicates that the Thy-1 antigenic determinant must be associated with the helper determinant in the same cell membrane for effective presentation to the immune system. It is unknown if this physical association of helper and Thy-1 represents linkage at the molecular level or if they are separate but joined by a membrane bridge. This observation differs fundamentally from the properties of helper sites controlled by the I-region which augment the induction of cytotoxic T cells¹⁰ in which help results, even when the two classes of antigen are expressed on separate cells. Taken together these studies indicate two pathways of antigen processing for T help to cellular antigens which are distinguished in their requirements for some form of structural association of the helper site and the determinant which is helped.

Stronger evidence for the immunological basis of the augmentation rendered by donor alloantigens was obtained by testing if this effect is thymus-dependent. From the results using 'B' mice (Table 3) it is apparent that this form of augmentation is thymus-dependent. The reduced overall magnitude of response is attributed to irradiation effects. The data also show that all the PFC detected on target cells of AKR produce specific anti-Thy-1.1 antibody since AKR/Cum target cells show no PFC.

Inbred strains of mice may differ for a multitude of alloantigens and those which are expressed on thymus cells are candidate helper determinants. Since it is known that structural associations between products of different genes can occur on the cell surface and that these intermolecular interactions are highly specific, for example between β_2 -microglobulin and H-2 or TL¹¹, it was possible therefore that help for Thy-1 was similarly associated with one or only a few select donor alloantigens controlled by their respective genes. To estimate the number of these genes, that is, of independently segregating genetic loci controlling the helper effect for Thy-1, a backcross experiment was done. A homogeneous population of AKR recipient mice was arranged into groups of two or three mice each and each group was inoculated with the thymus cells from individual (CBA × AKR/Cum) F_1 × AKR/Cum backcross progeny.

Since the F_1 hybrids are highly immunogenic it was expected that segregation of the helper loci of CBA would occur upon backcross mating the F_1 to the poorly immunogenic parent. Thymus cells from 76 backcross progeny mice of three weeks of age were injected into AKR recipients. Other recipients were injected with thymus cells from AKR/Cum and (CBA × AKR/Cum) F_1 donors which served as negative and positive controls respectively. Thus all donor mice were homozygous for the Thy-1.2 determining allele and segregants differed in their expression of putative helper alloantigens from the CBA parent.

The results in Fig. 1 show that the backcross progeny distribute into two categories of immunogenicity, those which fall into the zone of the poorly immunogenic AKR/Cum donors and those well above this zone and are thus augmented. Of 76 backcross mice, 23.7% (18 mice) were of the low immunogen type and 76.3% (58 mice) were augmented immunogens as compared with controls. This result is statistically consistent with the presence of two unlinked chromosomal loci in the donor CBA genome, each capable of augmenting in antigenic thymus cells the immunogenicity of Thy-1.2 for primary antibody responses (chi-square test $0.95 < P < 0.90$). The scattergram also suggests that the two 'helper loci' may differ in strength and that their representative genes are fully penetrant and dominant or codominant. It is unknown if the determinants which help the Thy-1 response are exclusive in their effect to Thy-1 or if they can also help other surface antigens. The identification of the helper determinants and their properties is therefore important to pursue.

There are indications that similar associative recognition phenomena may apply to responses to other surface antigens. In a recent study it was shown that, even subsequent to extraction and purification, a potent carrier effect of one cell surface molecule for another with which it natively associates on the cell membrane is preserved¹². The requirement for help of one surface antigen from other antigens may explain why most congenic immunisation regimens fail to produce anti-Ly antibodies¹³ as well as the absence of IgG production in a congenic H-2 immunisation¹⁴. In successful congenic immunisations the response may be more thymus-independent^{2,3} or alternatively obtain T cell help from antigens coded for by closely-linked genes. There is also evidence that some helper determinants may themselves be latent and are immunogenic only in the presence of other antigens¹⁵.

In summary the results show that the Thy-1 antigen is much more immunogenic if presented on donor cells which also carry other alloantigens and that this helper effect is thymus-dependent. These helper sites and the Thy-1 antigen must be expressed on the same cell surface and not on separate cells, indicating

Table 3 Evidence that helper alloantigens must be on the cells expressing the determinants which are helped and thymus-dependence of the effect

Expt 1		Anti-Thy-1.1 PFC per spleen (s.e.m.)
Thymus donor	Thy-1 phenotype	
AKR	Thy-1.1/1.1	345 (127)
B10.BR	Thy-1.2/1.2	0 (0)
(AKR×B10.BR)F ₁	Thy-1.1/1.2	4,554 (425)
AKR+B10.BR ^a	Thy-1.1/1.1	387 (72)
	+Thy-1.2/1.2	
AKR+B10.BR ^b	Thy-1.1/1.1	186 (36)
	+Thy-1.2/1.2	
Expt 2		Anti-Thy-1.1 PFC per spleen (s.e.m.)
Thymus donor	Pre-irradiation treatment	
AKR+B10.BR	Thymectomy	54 (71)
(AKR×B10.BR)F ₁	Thymectomy	9 (20)
AKR+B10.BR	Sham thymectomy	33 (22)
(AKR×B10.BR)F ₁	Sham thymectomy	*753 (252)

(AKR/Cum×CBA)F₁ mice, in groups of five recipients each, were injected i.v. with 4×10^7 of the indicated cell suspension except for the cell mixture where in (a) 8×10^7 cells were injected and in (b) 4×10^7 cells were injected. Cells were mixed in a 1:1 ratio. PFC were determined after 7 d. In Experiment 2 (CBA×AKR/Cum)F₁ mice were thymectomised or manipulated surgically but not thymectomised. After a recovery interval of 3 weeks all mice were given 750 rad of γ -irradiation and 5×10^6 fetal liver cells from syngeneic mice. The mice were used after an additional 5-week period.

* Portions of these spleens (1/30) were tested on AKR/Cum target and no PFC were detectable.

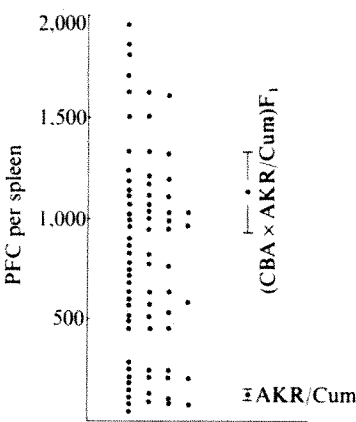


Fig. 1 Distribution of immunogenicity of thymus cells from individual backcross mice with segregating helper determinants. Evidence for two antigenic 'helper genes'. A large age-matched pool of AKR female mice was randomised and arranged in groups of two or three recipients per backcross donor thymus and in groups of five or six for control thymus suspensions (a pool of several donors). The recipients were immunised with 4×10^7 (CBA×AKR/Cum)F₁×AKR/Cum backcross thymus cells i.v. and PFC per spleen determined on day 7. Each point represents the mean PFC per spleen of two or three recipients. Vertical bars represent ± 1 s.e.m.

that some form of physical bridge between Thy-1 and its helper is important in the presentation of the surface antigen to the immune system similar to hapten-carrier antigens. Further, we have shown that highly effective helper sites are controlled by chromosomes lacking the relevant Thy-1 antigen and the data suggest that only two genetic loci control these helpers in primary antibody responses.

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Adenylate cyclase in a fibroblast mutant defective in glycolipid and glycoprotein synthesis

THERE is currently wide interest in the biological role of glycolipids and glycoproteins of the cell membrane. As some transformed cells have reduced levels of the more complex gangliosides and neutral glycolipids (see refs 1–3 for reviews), it has been suggested that complex gangliosides and particularly ganglioside G_{M1} may have an important role in regulating cell growth and in the responsiveness of adenylate cyclase (ATP pyrophosphate-lyase (cyclising), EC 4.6.1.1) to regulatory factors^{3,4}. The ganglioside G_{M1} is thought to be required for the activation of plasma membrane adenylate cyclase by cholera toxin; the toxin activates adenylate cyclase⁵ and binds specifically to ganglioside G_{M1} (refs 6–8). To investigate the effect of altered glycolipid and glycoprotein composition on adenylate cyclase activity we have used cell line AD6, a mutant of BALB/c 3T3 cells that is deficient in glucosamine phosphate acetyl transferase^{9–12}, and therefore partially blocked in its ability to synthesise complex carbohydrates, glycoproteins and glycolipids. Our results show that AD6 cells have a decreased responsiveness to cholera toxin, indicative of an altered membrane ganglioside composition, yet the responses of the enzyme to GTP, adrenaline, prostaglandin E_1 and fluoride are unaltered. Our results indicate that decreased synthesis of complex gangliosides does not result in alterations in adenylate cyclase other than a decreased response to cholera toxin.

For the present studies cells were grown in Dulbecco–Vogt's modified Eagle's medium supplemented with 10% calf serum (Colorado Serum) as described by Pouyssegur and Pastan⁹. For the determination of cholera toxin activation of adenylate cyclase cells were changed to serum-free medium 12 h before addition of cholera toxin. Cells were then incubated with the indicated concentration of cholera toxin (Schwarz-Mann) for 3 h

Fig. 1 Effect of cholera toxin treatment of intact Swiss 3T3 (●), BALB/c 3T3 (Δ), SV40-BALB (○), or AD6 (▲) cells on adenylate cyclase activity measured in a crude membrane fraction prepared from the indicated cell type. Cells were changed to serum-free Dulbecco–Vogt modified Eagle's medium and incubated with the indicated concentration of cholera toxin for 3 h before determination of adenylate cyclase activity. Enzyme activity was measured as described¹³ with 0.2 mM ATP, 3 mM Mg^{2+} and 1 mM EGTA.

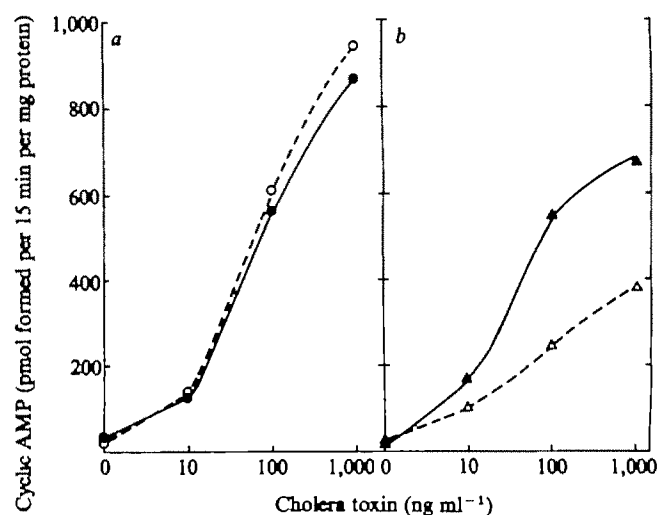
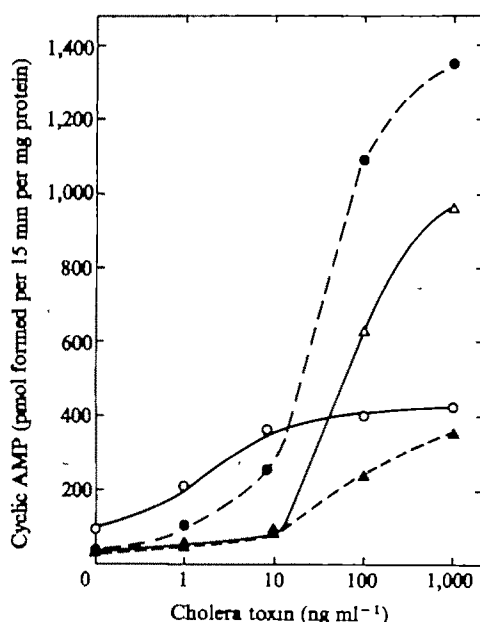


Fig. 2 Effect of G_{M1} on cholera toxin activation of adenylate cyclase in wild-type BALB/c 3T3 (a) or AD6 cells (b). Cells were incubated for 18 h in serum-free medium with (closed symbols) or without (open symbols) 50 pmol G_{M1} per dish, then washed three times with phosphate-buffered saline and once with serum-free growth media. Cells were then incubated in serum-free media with the indicated concentration of cholera toxin for 3 h before determination of adenylate cyclase activity as described in Fig. 1.

before collection. A crude membrane fraction was prepared and adenylate cyclase activity measured as described elsewhere¹³. Triplicate determinations of adenylate cyclase activity were routinely within $\pm 10\%$.

The block in the formation of *N*-acetylglucosamine and derived amino sugars would be expected to lead to decreased synthesis of the more complex gangliosides from ganglioside G_{M3} (see the pathway for ganglioside biosynthesis presented in ref. 3) as well as glycoproteins. From published data^{14,15} it is established that Swiss 3T3 cells have higher levels of the complex gangliosides than do BALB/c 3T3 cells, and SV40-transformed cells have very low or undetectable amounts of G_{M1} and G_{D1a} . Data in Fig. 1 show that incubation of intact cells for 3 h with cholera toxin markedly enhances basal adenylate cyclase activity. Swiss 3T3 cells which contain more of the complex gangliosides are more sensitive to cholera toxin activation presumably due to G_{M1} functioning as the toxin receptor. AD6 cells have a reduced response to cholera toxin. This result suggests that AD6 ganglioside levels are comparable to the SV40-transformed cells.

A chemically transformed line of mouse fibroblasts has been described which lacked G_{M1} and was unresponsive to cholera toxin (ref. 16). When incubated in medium containing G_{M1} , these cells bound the ganglioside and responded to cholera toxin as measured by activation of adenylate cyclase and elevation of cellular cyclic AMP content^{16,17}. To determine if the incorporation of G_{M1} into AD6 or wild-type BALB/c 3T3 cells would improve the response to cholera toxin, confluent cells were changed to serum-free growth medium and incubated with 50 or 500 pmol G_{M1} per dish (100 mm dish containing 5 ml growth media) for 12 h. The ability of cholera toxin to enhance adenylate cyclase activity was determined as described in Fig. 2. A few of the cells (<15%) detached from the substratum during incubation in serum-free medium; only those cells remaining on the dish were used to determine cholera toxin responsiveness. When AD6 cells were treated with 50 or 500 pmol G_{M1} per dish, a significant increase in cholera toxin activation was observed (Fig. 2). Thus, the incorporation of G_{M1} into the mutant membrane corrects the inability of cholera toxin to activate adenylate cyclase of the mutant to the same extent as observed with the wild-type parent. Similar treatment of the BALB/c 3T3 parent did not result in further stimulation by cholera toxin.

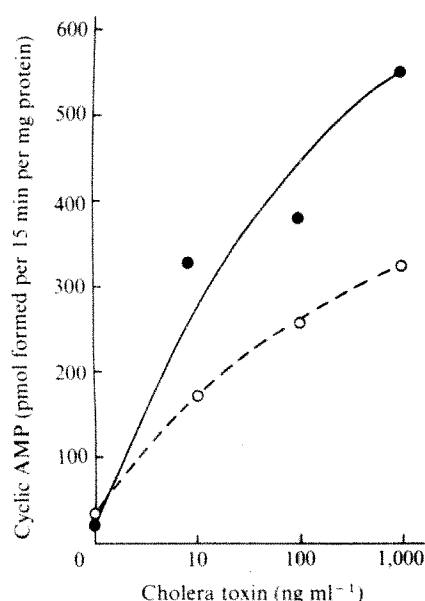


Fig. 3 Effect of *N*-acetylglucosamine on cholera toxin activation of adenylate cyclase in AD6 cells. Cells were grown to confluence in the presence (●) or absence (○) of 10 mM *N*-acetylglucosamine. Addition of the indicated concentration of cholera toxin and determination of enzyme activity were carried out as described in Fig. 1.

Keenan *et al.*¹⁸ have suggested that artificially supplied gangliosides assume a configuration or localisation in the membrane different from that of the native ganglioside. Nevertheless, the results of Fig. 2 show that sufficient ganglioside G_{M1} molecules become associated with the cell surface to serve as receptors for cholera toxin. In addition to showing that ganglioside G_{M1} may act as a cholera toxin receptor, these results emphasise that the low cholera toxin response observed in AD6 cells is due to a decreased ability to recognise and bind cholera toxin.

Previous studies have demonstrated that when AD6 cells are grown with *N*-acetylglucosamine the defect in the *N*-acetylation of glucosamine-6-phosphate is bypassed and the mutant cells revert to the wild-type phenotype^{10,11}. When AD6 cells were grown with 10 mM *N*-acetylglucosamine, the ability of cholera toxin to stimulate adenylate cyclase was enhanced (Fig. 3). This result, together with the ability of exogenous G_{M1} to increase cholera toxin activation of adenylate cyclase in AD6, indicate that it is the altered carbohydrate composition of the plasma membrane of AD6 cells that accounts for their decreased sensitivity to cholera toxin. As AD6 cells have normal growth

Table 1 Adenylate cyclase activities of wild-type, AD6 and transformed BALB/c 3T3 cells

Addition	Wild-type BALB/c 3T3	Adenylate cyclase activity		
		AD6	Kirsten- BALB	SV40- BALB
	cyclic AMP (pmol formed per 10 min per mg protein)			
None	31 ± 4	25 ± 3	92 ± 6	101 ± 6
GTP	35 ± 4	29 ± 4	272 ± 9	319 ± 15
GTP + L-adrenaline	378 ± 17	336 ± 20	269 ± 14	346 ± 16
GTP + PGE ₁	89 ± 7	69 ± 7	295 ± 21	352 ± 29
Fluoride	521 ± 27	452 ± 31	1052 ± 46	1138 ± 22

Adenylate cyclase activity was measured as described elsewhere¹³ with 0.2 mM ATP, 3 mM MgCl₂, 1 mM EGTA, and 10 μM GTP, 10 μM L-adrenaline, 10 μg ml⁻¹ prostaglandin E₁ or 10 mM NaF as indicated. A crude membrane preparation of each cell type was used as the source of enzyme. Values presented are the mean ± S.E. for duplicate and triplicate determinations.

properties, this result also indicates that the decreased ganglioside content found in some transformed cells is not by itself a primary cause of the transformed state.

In studies with several virally transformed fibroblast lines a defective adenylate cyclase system has been observed^{19,20}. It is evident from the results presented in Table 1 that BALB 3T3 cells transformed by either the Kirsten sarcoma virus or SV40 virus have elevated basal, GTP-stimulated and fluoride-stimulated adenylate cyclase activities and a decreased or absent response to hormonal activation. Yet, with AD6 cells the adenylate cyclase activity was fully responsive to hormonal stimulation. This result tends to suggest that changes in membrane structure other than modification of the carbohydrate moieties are responsible for the altered properties of adenylate cyclase noted with transformation.

The mutant cell AD6 used in this study has also been found to have decreased binding of epidermal growth factor, presumably because its receptor is a glycoprotein²¹. The use of mutants with defined defects such as AD6 should help elucidate the role of glycolipids, glycoproteins and other complex molecules in cell behaviour.

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Release of acetylcholinesterase from rat hemidiaphragm preparations stimulated through the phrenic nerve

ACETYLCHOLINESTERASE (AChE; EC 3.1.1.7) is known to be transported rapidly within peripheral motor nerves, both towards and away from nerve cell bodies^{1–3}. Of the total amount of neural AChE, approximately 15% is mobile, with about 10% moving towards the nerve terminals and about 5% returning towards cell bodies, the remaining 85% of the enzyme being stationary. Since the mean velocity of orthograde transport is about twice that of the retrograde flow, the total flux of enzyme is 3–4 times greater in the distal than in the proximal direction. Although the fate of this transported

AChE is not entirely clear, it has recently been shown that some of the distally transported enzyme may never arrive at the nerve terminals, being incorporated into the axolemma at sites all along the neurone^{4,5}. This axolemmal incorporation is slow, and it seems likely that AChE would accumulate in the terminals unless there were local mechanisms for its removal, such as proteolytic digestion or extrusion from the cell as has been demonstrated in non-neuronal tissue^{6,7}. We now offer evidence that AChE is released from nerve terminals in response to nerve stimulation and suggest that this release may be parallel to, but separate from, release of neurotransmitter.

Isolated rat phrenic nerve-hemidiaphragm preparations were incubated at 37°C in beakers each containing 1 ml of oxygenated physiological saline solution with (+)-tubocurarine (1.4×10^{-5} M) added to prevent muscle contraction. Preparations were equilibrated in this solution for 1 h, followed by three 15-min incubation periods. At the end of each incubation period, preparations were transferred to new beakers containing fresh medium. The media exposed to tissue during experimental periods were chilled to -20°C immediately after collection and were subsequently analysed for their AChE content by the radiometric assay of Potter⁸. Resting release of AChE was determined from the enzyme activity in the media collected after the first and second experimental periods. This activity averaged 39.2 ± 1.5 nmol of acetylcholine (ACh) hydrolysed per h per ml of medium (mean $\pm$ s.e.m., $n=98$). During the third experimental period, the phrenic nerve was gently lifted out of the bathing medium and stimulated through platinum electrodes with supramaximal 5-V square-wave pulses of 0.4 ms duration and at frequencies of 0–200 Hz. The nerve was positioned such that the oxygenation of the bath splashed medium on the nerve and kept it moist during the stimulation period. To determine the effects of stimulation, resting release (mean activity collected during the first two experimental periods) was subtracted from the activity accumulating during the third period of each experiment. Release from preparations not stimulated during the third period was essentially identical to resting release, whereas stimulation increased the release of AChE in a frequency-related manner (Fig. 1). Stimulation-induced release was statistically significant at 0.2 Hz and highly significant at frequencies of 2 Hz or above, even though the maximum release (at 200 Hz) was only 32% above basal levels (Fig. 1). Since the concentration of (+)-tubocurarine used in these experiments

totally prevented muscle contractions, and has been shown to eliminate muscle action potentials in response to nerve stimulation⁹, it is unlikely that the AChE released from our stimulated preparations arose from the muscle. In two experiments in which we incubated 2-cm lengths of phrenic nerve from which the diaphragm had been removed by a cut close to the muscle, we could detect neither resting release nor release in response to 20-Hz stimulation. We infer from these data that little or no AChE was extruded from non-terminal axons.

Low Ca^{2+} -high Mg^{2+} solutions have been shown to inhibit nerve-stimulated release of the neurotransmitter ACh^{10,11}. In our experiments, resting release of AChE was somewhat reduced in the presence of low Ca^{2+} and high Mg^{2+} (mean = 18.1 ± 1.7) and nerve-stimulated release of AChE at a frequency of 0.2 Hz was completely inhibited (Fig. 1). Although these results suggest a similarity between ACh release and AChE release, there is one important difference. The latter was found to be frequency related up to 200 Hz, whereas ACh release has been reported not to be related to frequency beyond 25 Hz¹². It therefore seems unlikely that the enzyme is extruded from the same sites or by the same mechanism as the neurotransmitter.

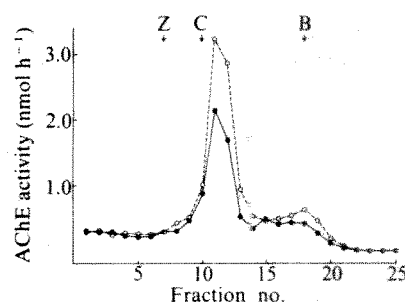


Fig. 2 Velocity sedimentation profile of AChE activity from phrenic nerve-hemidiaphragm effluent. The bathing media of nerve-muscle preparations were layered on 4.8 ml density gradients of 10–40% (w/v) sucrose containing 1 M NaCl, 0.2 mM EDTA, 50 mM Tris-HCl (pH 7.4) and 1% Triton X-100, and were centrifuged at 58,000 r.p.m. for 16 h in an SW65 rotor in an L2 65B ultracentrifuge. AChE activity from resting (●) and 2.0 Hz nerve-stimulated (○) preparations was assayed as described and corrected for effects of the sucrose. Marker proteins used to calibrate the gradients were 16S β -galactosidase (Z); 11.2S catalase (C); and 4.4S bovine serum albumin (B). AChE activity in the 10S (fractions 9–13) and 4S (fractions 16–20) peaks of nerve-stimulated preparations differed significantly ($P < 0.05$) from the corresponding peaks in the resting state as determined by a paired t -test. The values represent the means of four nerve-muscle preparations for both resting and nerve-stimulated tissues.

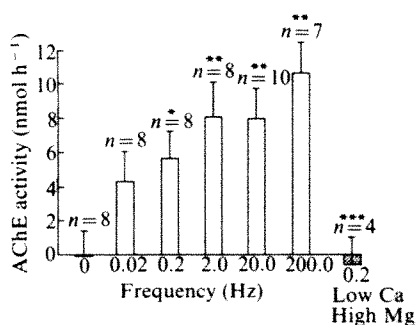


Fig. 1 Release of AChE from nerve-stimulated rat phrenic nerve-hemidiaphragm preparations. Tissues were incubated in isotonic buffer of the following composition (mM): NaCl, 115.0; KCl, 5.0; CaCl_2 , 2.5; MgCl_2 , 0.5; glucose, 5.0; sucrose, 24.0; HEPES (pH 7.4), 15.0; bovine serum albumin, 0.2%. Low Ca^{2+} -high Mg^{2+} buffer differed in the following constituents: CaCl_2 , 0; MgCl_2 , 10.0; sucrose, 3.0. Accumulations of AChE in response to nerve stimulation represent activity in excess of the resting release of enzyme (see text). Mean values and s.e.m. are shown. Significance levels, determined by Student's t -test, are: * $P < 0.05$ at 0 Hz; ** $P < 0.005$ at 0 Hz; *** $P < 0.001$ at 0.2 Hz in normal buffer. A least squares regression analysis showed a significant linear relationship between release of AChE and log frequency over the range 0.02–200 Hz (slope = 1.46 ± 0.58 , $P < 0.02$).

Three isozymes of AChE have been identified in mammalian muscle and nerve, having sedimentation coefficients of approximately 4S, 10S and 16S (refs 13–15). We studied the molecular forms of AChE released into the bathing medium by resting and stimulated nerve-muscle preparations. Tissues were incubated as described above; however, to concentrate the enzyme, the volume was reduced to 0.5 ml and the collection periods were increased to 30 min. Aliquots (0.2 ml) of bathing medium were layered on to sucrose density gradients, centrifuged, fractionated from the bottom of the tube, and assayed for AChE (Fig. 2). The media collected from resting preparations were found to contain both 4S and 10S enzyme, but about three times more of the latter than of the former. Both forms of AChE were released by stimulation, but more than 80% of the released enzyme sedimented at 10S. No 16S AChE was detected in media from resting or stimulated preparations.

The distribution of molecular forms of AChE released into the bathing media from our nerve-muscle preparations does not resemble the distribution in muscle, which contains significant amounts of 16S enzyme, primarily in the endplate

regions, and approximately equal amounts of 10S and 4S enzyme^{13,14}. The distribution of released forms of AChE is, however, similar to the distribution in nervous tissue^{13,15} and resembles especially closely the distribution of forms carried distally by rapid transport along peripheral nerve axons¹⁵. These results suggest not only that the AChE released following nerve stimulation derives from the nerve terminal, but that much of the resting release of AChE also comes from nerves rather than from muscle.

Several investigators have demonstrated that substances other than neurotransmitters may be released from motor nerves on electrical stimulation. Amino acids¹⁶, as well as proteins^{17,18} presumably transported distally from cell bodies, are released from nerve terminals. Although AChE has not previously been identified as being released from nerve-muscle preparations, it has been shown to be released from cultured chick embryo spinal cord cells¹⁹ and from adrenal glands bathed in high potassium solutions^{20,21}. In the latter case, the release was shown to depend on calcium and was believed to arise, at least partly, from splanchnic nerves²¹.

The content of AChE in muscle is influenced, in part, by the motor neurone²²⁻²⁴. This has been demonstrated convincingly by the reduction of AChE activity in denervated muscle. Although denervated muscle is still capable of synthesising AChE, the enzyme levels never reach those of innervated controls²⁵. In addition, blockade of axonal transport in motor nerves with colchicine has produced denervation-like changes in the AChE content of muscles without interrupting neuromuscular transmission²³. These results suggest that muscle AChE is partially regulated by substances that are transported down the nerve and are released from the nerve terminals.

Our results do not exclude the possibility that one of these regulatory substances is AChE itself. The enzyme has not been demonstrated to be associated with synaptic vesicles, but has been shown to be localised in cisternae of smooth endoplasmic reticulum (SER) in nerves²¹. We suggest that these cisternae may release their contents when action potentials invade the nerve terminals. The mechanism of this release could share features in common with the exocytotic release of neurotransmitter from the synaptic vesicles. In any case, if the released AChE were able to diffuse across the synaptic cleft, it might be responsible for some of the trophic effects of nerves on muscle and could even serve as one source of muscle AChE.

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Evidence for two separate opiate peptide neuronal systems

WHEN Hughes *et al.*¹ first described the sequences of the enkephalins, they pointed out that methionine-enkephalin (met-enkephalin) could be found in β -lipotropin (β -LPH 61-65). The entire C-terminal fragment of β -LPH was subsequently found to have opiate-like properties (β -LPH 61-91 or β -endorphin)²⁻⁷, and it seemed reasonable to hypothesise that the primary biosynthetic route for met-enkephalin was β -LPH to β -endorphin and eventually to met-enkephalin. Although this hypothesis awaits biochemical evidence, immunocytochemical studies of these peptides may shed light on the question. If β -LPH and β -endorphin are, in fact, enkephalin precursors which are stored by enkephalinergic neurones, one would expect them to be visualised in met-enkephalin-positive cells. Several laboratories produced immunocytochemical maps of the distribution of enkephalin-like-immunoreactivity (enkephalin-reactivity) in mammalian brain⁸⁻¹¹. Watson *et al.*¹² demonstrated β -LPH-reactive cells and processes in rat brain. This β -LPH reactivity has the same distribution as β -endorphin-like-immunoreactivity (β -endorphin-reactivity) as reported by Bloom *et al.*¹³ and Watson *et al.*¹⁴. However, the distribution of the β -LPH/ β -endorphin-reactive system on the one hand and the met-enkephalin-reactive system on the other seemed quite different. Met-enkephalin-reactivity was seen in many local cell groups throughout the neuraxis, especially the lower brain stem⁸⁻¹¹, whereas β -LPH- and β -endorphin-reactivities were found only in a single hypothalamic cell group with long ascending and descending processes¹²⁻¹⁴. We present here biochemical and anatomical evidence for the existence of two separate opioid systems in brain—an enkephalin system and a β -LPH/ β -endorphin system. We also present immunocytochemical data for the coexistence in the brain of ACTH with β -LPH/ β -endorphin.

The investigation of the coexistence of ACTH with β -LPH is based on evidence from the pituitary where β -LPH and β -endorphin have been localised in the corticotrophs^{15,16}, in fact, within the same granules¹⁷. These two pituitary peptides possess a common precursor of molecular weight, 31,000 as demonstrated in cell cultures¹⁸, and, in the intact rat, are co-released into the plasma upon stress¹⁹. Such evidence, coupled with the biochemical and immunocytochemical demonstration in brain of β -LPH (refs 12, 20, 21) and of ACTH (refs 22, 23), has led us to investigate whether the same relationship between ACTH and β -LPH/ β -endorphin exists in brain as it does in the pituitary.

Because the present studies depend primarily on the use of multiple antibodies with unique cross-reactivities, the following is a summary of their characteristics. Four antisera were used: anti-methionine-enkephalin (our laboratory), anti- β -LPH (from C. H. Li) anti-ACTH and anti- β -endorphin (R. Mains/B. Eipper). Cross-reactivity of these antisera was tested both in radioimmunoassay (RIA) and immunocytochemical systems by classical competition methods. The met-enkephalin antibody has been described elsewhere²⁴. It exhibits no cross-reactivity with β -LPH, β -endorphin, ACTH or β -melanocyte stimulating hormone (β -MSH). Its cross-reactivity with leu-enkephalin is less than 1:200 and with α -endorphin less than 1:250. ACTH antibody was affinity-purified using ACTH 1-24 (ref. 25) and has been shown to be directed against ACTH 11-24. It does not cross-react with β -LPH, β -endorphin, or

their smaller fragments (β -MSH and met-enkephalin). β -Endorphin antiserum is directed against α -endorphin (β -LPH 61-76). It cross-reacts with β -endorphin and with the full β -LPH molecule on an equimolar basis. It does not, however, cross-react with met-enkephalin, ACTH or β -MSH. β -LPH antiserum seems to be primarily directed at the N-terminus of the β -LPH molecule, as it recognises β -LPH (1-41) and does not recognise β -MSH (β -LPH 41-58)^{26,27}. In the conditions of our RIA this antiserum exhibits very low cross-reactivity with β -endorphin (less than 1:100) with a very shallow dilution curve. In fact, we cannot obtain more than 10% inhibition of binding with concentrations of β -endorphin at 100 nM, whereas β -LPH causes 10% inhibition at 0.05 nM. We have carried out extractions of rat pituitary and brain which are optimal for β -LPH with little contamination with β -endorphin. The β -LPH antiserum demonstrates activity against these ' β -LPH extracts'. The dilution curves are not parallel to those of β -endorphin in this RIA, suggesting that we are not measuring the C-fragment of β -LPH, but possibly its N-terminus. It should be noted, however, that β -LPH N-terminus varies with phylogeny and has not been sequenced in the rat.

Finally, some of the immunocytochemical studies using β -LPH antiserum were carried out in the presence of excess β -endorphin (1 μ M) and the results were unaltered. In contrast, the addition in much lower concentrations of β -LPH (5 nM) led to complete blockade of the anatomical demonstration. The low cross-reactivity of this antiserum with β -endorphin, the different slopes of dilution curves and the lack of blockade by β -endorphin in anatomical studies suggest that we are looking primarily at the N-terminus of β -LPH, and that the cross-reactivity with C-fragment is due to a small sub-population of antibody.

Thus, three of the antisera used in our studies apparently recognise three different aspects of the precursor molecule; that is, ACTH, the N-terminus of β -LPH, and β -endorphin (or C-terminus of β -LPH). A positive response of all three within the same system might indicate the existence within that system of either precursor or of its individual components.

The evidence for two separable opioid systems is derived from both anatomical observations and a lesion study. As mentioned above, only one hypothalamic cell group combines β -LPH and β -endorphin reactivity, whereas met-enkephalin-positive cells can be visualised in several regions throughout the brain stem but not in the β -LPH/ β -endorphin-containing hypothalamic group. Furthermore, some brain areas are rich in met-enkephalin axons and terminals (for example, globus pallidus), but are almost totally devoid of β -LPH/ β -endorphin activity.

To ascertain that these differential distributions are not due to any artefact of visualisation, we have devised a technique for extracting β -LPH and the enkephalins from the same tissue^{21,28}. As the structure of β -LPH in the rat is unknown, and as our extract dilution curves differ in slope from the human β -LPH curve, all values are expressed in units as described in Table 1. Using the β -LPH and met-enkephalin RIAs, we obtain very different distributions of the two peptides in various brain regions. Again, the striatum has the highest concentration of met-enkephalin in the brain (1,750 pmoles per g wet weight), but β -LPH levels in the striatum (1 unit per g) are about 10-fold lower than in the hypothalamus (9-10 units per g) and are exceeded by levels in the central grey and thalamus (2.5 units per g).

We therefore reasoned that if the two systems are in fact separate, specific lesions may be able to differentiate between them. We produced unilateral lesions of the basal hypothalamus arranged so as to destroy the majority of the β -LPH-positive cells ($n=5$). The control group was sham operated ($n=5$). Ten days after surgery, the animals were killed, their brains rapidly removed and the cortex, septum, cerebellum and hippocampus dissected away. The remainder of the brain was divided into a posterior region containing the medulla-pons, central grey, tegmentum and colliculi, and an anterior region

Table 1 Effect of unilateral hypothalamic lesions on met-enkephalin and β -LPH in two regions

	Enkephalin (pmol per g tissue) Met-enkephalin	β -LPH: (units per g) β -LPH
Posterior region	Controls = 1,110 $\pm$ 193 Lesioned = 890 $\pm$ 230 %C = 80% Not significant	Controls = 5.0 $\pm$ 0.4 Lesioned = 2.3 $\pm$ 0.8 %C = 45% $P < 0.02$
Anterior region	Controls = 1,431 $\pm$ 85 Lesioned = 1,159 $\pm$ 165 %C = 81% Not significant	Controls = 2.5 $\pm$ 0.4 Lesioned = <1.2 %C = <50%

Lesions were placed in the basal hypothalamus unilaterally to destroy β -LPH cells. Brains were rapidly removed, dissected, frozen and homogenised with 0.4 M PCA. The supernatant contained met-enkephalin and the pellet β -LPH which were assayed by specific RIAs. The anterior region includes hypothalamus, thalamus and striatum. The posterior region includes central grey, medulla pons, tegmentum and colliculi. As lesions are unilateral, a 50% drop could indicate complete loss on one side (see text). A similar study of a lesion in the periaqueductal central grey produced major changes in met-enkephalin with minor changes in β -LPH levels. β -LPH values are expressed in units. This is necessary because the dilution curve of rat extracts is not parallel to human β -LPH standards. The quantitation is carried out in terms of a dilution curve of pituitary rat extract. At 50% inhibition, 1 unit corresponds to 1 pmol of human β -LPH.

involving thalamus, hypothalamus and striatum. The brains were rapidly frozen on dry ice, and β -LPH and enkephalin were extracted using 0.4 M perchloric acid as described elsewhere^{21,28}. Table 1 shows the results of β -LPH and enkephalin RIAs. As can be seen, β -LPH levels decreased significantly to about 50% of control, a maximal drop for a unilateral lesion. On the other hand, met-enkephalin levels showed a lower drop which did not reach significance in either brain part. It is important to note that such a differential effect cannot be attributed to differences in the turnover rates of the two substances, because in similar conditions lesions in the mesencephalic central grey produce the opposite pattern (that is, a significant decrease in enkephalin and no change in β -LPH levels in the anterior region)^{21,28}. The sites of lesions in these studies were immunohistologically confirmed in a separate group of animals.

As the above studies suggested the separability of β -LPH/ β -endorphin from the enkephalin systems, we proceeded to characterise the endorphin system further. Using the above antisera, we have studied the anatomical distribution of β -LPH, β -endorphin and ACTH in the same section of rat brain.

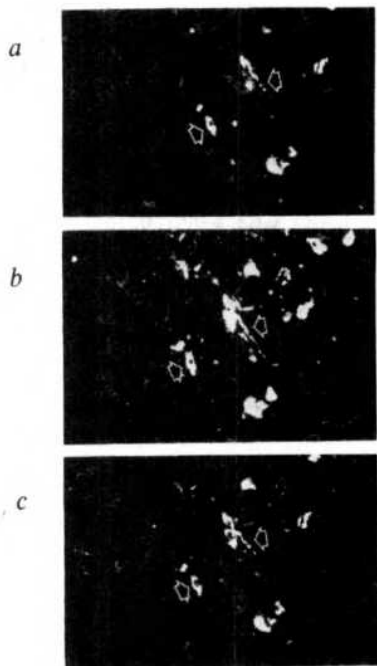
Male Sprague-Dawley rats were injected intracerebroventricularly with 50 μ g colchicine in 50 μ l of saline 48 h before death. Sections were prepared for immunocytochemistry as described elsewhere^{8,9}. Briefly, the rats were perfused with 4 g per 100 ml formaldehyde, their brains removed and blocked, re-exposed to formaldehyde solution followed by 10 g per 100 ml sucrose phosphate-buffered saline (PBS), frozen in liquid nitrogen and then sectioned in a -20°C cryostat. 10- μ m sections were incubated for 48 h with rabbit anti-ACTH antibody (1/1,500). They were washed and exposed to goat anti-rabbit-fluorescein isothiocyanate (FITC) tagged IgG (1/80), rewashed, viewed and photographed with a Leitz Orthoplan microscope. The sections then had their coverslips removed, were washed and exposed to 6 M guanidine HCl overnight (to break antigen-antibody bonds); their coverslips were then replaced, and they were viewed again. The areas photographed earlier were exposed to ultraviolet (UV) light until the residual fluorescence was completely exhausted, and then rephotographed. The section was washed again, and incubated with goat anti-rabbit IgG (without FITC) to block any remaining rabbit anti-ACTH antibody which had not been removed by guanidine HCl. The section was then exposed to the second antibody (β -endorphin) and the entire sequence repeated. The

third antibody (β -LPH) was visualised in the same way. Thus, exactly the same area could be studied for immunoreactivity to ACTH, β -endorphin and β -LPH.

All three antisera could be blocked by 1 μ M of the appropriate peptide. Several sections were run to demonstrate that goat anti-rabbit-FITC did not rebind to residual primary antibody, that guanidine HCl did not damage the immunoreactivity of these three peptides, that after guanidine HCl an antigen could be revisualised, and that guanidine treatment is not necessary for the demonstration of the second or third antigen. Further, some animals were run without colchicine to ascertain that this drug did not alter the pattern of immunoreactivities. These sections showed the same results, although cell body fluorescence was less intense.

Sections through the periaruate region of the hypothalamus were studied because cells for all three peptides (ACTH, β -LPH and β -endorphin) were found only in this area. Figure 1 shows the ACTH-positive (1a), β -endorphin-positive (1b) and β -LPH-positive (1c) cells in the arcuate region. All three antisera demonstrated the same cells and fibres. We observed no cells which contained one peptide and not the others. Fibres in the hypothalamus, medial amygdala and periventricular nucleus of the thalamus exhibited the same pattern for all three antisera. Fluorescence was absent in peptide-blocked (1 μ M) controls taken from serial sections (not shown). We have not yet seen any overlap between cells positive for the enkephalin system and those positive for ACTH/ β -LPH/ β -endorphin.

Fig. 1 Immunocytochemical demonstration of ACTH, β -endorphin and β -lipotropin immunoreactivity in the same hypothalamic neurones. Adult male Sprague-Dawley rats given 50 mg colchicine intracerebroventricularly, 48 h earlier; prepared for immunocytochemistry as in text. *a*, ACTH-positive cells in the periaruate region of hypothalamus (see arrows). Magnification $\times 200$. Fluorescence was completely lost after exposure to 6 M guanidine HCl and UV light. *b*, The same section, after exposure to 6 M guanidine HCl and UV light, was re-exposed to goat anti-rabbit IgG to block remaining rabbit IgG. It was then exposed to β -endorphin antiserum. The same cells are revisualised (see arrows). Magnification $\times 200$. Again, exposure to 6 M guanidine HCl and UV light eradicated fluorescence. *c*, Re-exposure to goat anti-rabbit IgG as in *b*, followed by incubation with β -LPH antiserum. The same cells and processes are seen in all three photographs. Excess peptide (1 μ M) blocked all fluorescence.



Using anatomical, biochemical and immunological tools, it has been possible to demonstrate the existence of two separate opiate peptide neuronal systems, one being an enkephalin system and the other a β -LPH/ β -endorphin/ACTH-positive system. During this work, Rossier *et al.*²⁹ reported that β -endorphin and enkephalin, as measured by RIA, are located in different proportions in several brain regions, again supporting the hypothesis of two separate opiate peptide systems. Although it is possible that β -endorphin serves as a precursor for met-enkephalin in enkephalin cells, it is also likely that it functions as a neuroregulator in its own neuronal pool. Further, it seems unlikely that met-enkephalin is purely a breakdown product of β -endorphin, as these peptides occur in morphologically separate systems and can be differentially altered by lesions. The relationships between these two neuronal-biochemical systems and between the peptides within each system remain to be determined.

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The role of GABA in the substantia nigra

STRIATONIGRAL GABAergic fibres are widely believed to exert a tonic inhibitory influence on the dopamine (DA)-containing cells of the substantia nigra (SN), and contraversive and ipsiversive circling behaviour can be explained in terms of a greater or lesser activity of the dopaminergic neurones, respectively^{1,2}. However, the blockade of γ -aminobutyric acid (GABA) receptors with intranigral picrotoxin occasionally stimulates rats to turn ipsiversively^{3,4}, rather than evoking the more commonly observed response in the opposite direction⁵. Similarly, injections of GABA agonists into the SN sometimes trigger an atypical contraversive turning syndrome^{3,4,6,7}. We describe here a study which sought to reconcile these paradoxical phenomena by investigating the effects on circling behaviour of decreasing the influence of GABA within the SN with picrotoxin, and of potentiating the action of GABA with *cis*-1,3-aminocyclohexane carboxylic acid (ACHC)⁸, a neuronal GABA uptake inhibitor. We show that the same drugs are capable of producing opposite turning responses when injected into the rostral and caudal parts of the SN. Moreover, behaviour patterns influenced by the anterior SN are accompanied by significant changes in homovanillic acid (HVA) levels in the striatum and require an intact DA system, whereas turning responses initiated by caudal drug injections are apparently not mediated by the dopaminergic nigrostriatal pathway.

Injections of picrotoxin (100 ng) into the rostral SN initiated immediate contraversive rotatory movements and a rise in striatal HVA concentration ipsilaterally, indicating that striatal DA utilisation had risen on the treated side of the brain (Fig. 1*a* and Table 1). By contrast, the administration of ACHC (10 ng) to the same area produced consistent ipsiversive circling and a drop in HVA formation in the corresponding striatum (that is, a fall in DA turnover). Control injections of physiological saline were never observed to alter the animals' behaviour or to modify striatal DA turnover. Groups of 12 rats receiving one or two doses of 6-hydroxydopamine (6-OHDA) showed, one week later, mean falls of 67.5% and 88.4%, respectively, in striatal DA levels. 6-OHDA treatment abolished the turning responses to both rostral picrotoxin and ACHC, and significant attenuation of both drug-induced behaviours was achieved with haloperidol (0.4 mg per kg, intraperitoneally) and electrocoagulation of the crus cerebri (Fig. 1*a*). From these data we conclude that the behavioural

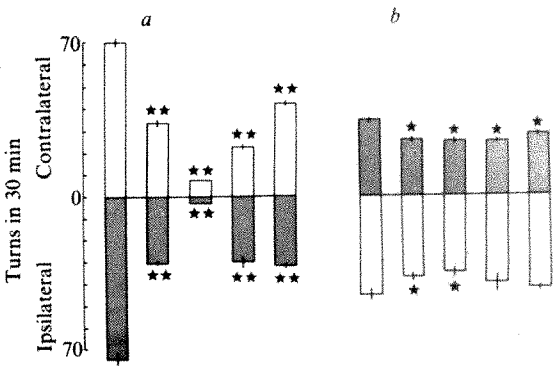


Fig. 1 Turning responses elicited by picrotoxin (open columns) and ACHC (shaded columns) injected into the rostral (*a*) and caudal (*b*) regions of the SN (see legend to Table 1). Results are shown for injections made into untreated control animals (first columns, *n* = 12), for groups of six rats pretreated with one (second columns) or two doses (third columns) of 6-hydroxydopamine unilaterally into the median forebrain bundle (4 μ g in 2 μ l, one week earlier), or haloperidol (0.4 mg per kg, intraperitoneally, 30 min previously) (fourth columns), and for rats with one-week-old unilateral electrolytic lesions of the crus cerebri (fifth columns, *n* = 6). Electrolytic lesions were made by implanting fine concentric bipolar electrodes into the crus cerebri close to the mammillary bodies and passing a direct current of 1 mA for 10 s. **P* < 0.01, ***P* < 0.001 compared to controls (Student's *t*-test).

responses to picrotoxin and ACHC, delivered discretely into the anterior region of the SN, were caused by modifying the levels of endogenously released GABA and thereby altering the activity of the DA cells.

We found it was also possible to make rats rotate by injecting either of these two drugs into the caudal area of the SN. Surprisingly, the direction of turning was now opposite to that evoked by rostral drug placements (Fig. 1*b*). We have no evidence that these caudally evoked responses involved the nigrostriatal DA system, as there were no corresponding changes in striatal HVA production (Table 1) and the behaviours were scarcely attenuated by successful lesions of the median forebrain bundle (Fig. 1*b*). Similarly, haloperidol only slightly reduced ACHC-induced contralateral rotation, whereas picrotoxin-induced ipsilateral movements were completely insensitive to DA receptor blockade. Interestingly, both drugs were more or less equally effective when administered to the caudal SN of crus cerebri-lesioned rats, suggesting to us that the GABA afferents to the anterior and posterior areas of this nucleus may follow separate paths.

The finding that the direction of turning elicited by intranigral injections of picrotoxin and GABA-like drugs depends on whether the compounds are delivered rostrally or caudally in the SN offers a simple explanation for the contrasting behavioural actions of these drugs that have been reported by different investigators^{4,7,12}. The traditional notion of an inhibitory link between GABA- and DA-containing neurones in the rostral SN therefore seems sound, but it does not account for the opposite action of GABA in the caudal SN. Two possible explanations warrant consideration. Where contraversive rotation induced by the intranigral administration of GABA-like drugs¹² can be related to increased nigrostriatal dopaminergic activity¹³, it may be conjectured that the compounds are acting indirectly by suppressing the activity of nigral inhibitory interneurones, thereby de-inhibiting (that is, exciting) the nigrostriatal DA pathway^{13,14}. On the other hand, we have found that behavioural responses elicited by modifications to the GABA system in the caudal SN are resistant to inhibition by procedures which attenuate nigrostriatal DA cell activity. We interpret this to mean that a second

Table 1 Influence of intranigral picrotoxin and ACHC on striatal HVA concentration

Drug treatment	Placement	Striatal HVA concentration (μ g per g wet weight)		<i>P</i>
		Contralateral	Ipsilateral	
Picrotoxin	Rostral	0.35 $\pm$ 0.02	0.69 $\pm$ 0.04	< 0.001
	Caudal	0.38 $\pm$ 0.02	0.36 $\pm$ 0.02	NS
ACHC	Rostral	0.39 $\pm$ 0.02	0.23 $\pm$ 0.02	< 0.001
	Caudal	0.41 $\pm$ 0.02	0.46 $\pm$ 0.02	NS

Male Wistar albino rats (Tuck, 190–210 g) were anaesthetised with halothane and injected stereotactically (0.1 μ l over 5 min) with picrotoxin or ACHC into the rostral (A2.5, L2, D-2) or caudal (A1.6, L2, D-2) regions of the SN⁹. On recovery, rats were placed singly in cages and the numbers of complete revolutions made in a minute were scored every 5 min for a total of 30 min. DA¹⁰ and HVA¹¹ were assayed fluorimetrically in corpora striata dissected from the brains of separate groups of rats injected intranigral with the drugs and killed after 15 min when the frequency of turning was highest. Each value is the mean $\pm$ s.e.m. of six separate experiments. NS, not significant by Student's *t*-test.

system of non-dopaminergic (excitatory?) neurones originates in the region of the SN and cooperates in the control of motor activity and that these, too, are under inhibitory GABA control.

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Striatal dopamine release and contraversive rotation elicited by intranigally applied muscimol

SEVERAL lines of evidence indicate that release of dopamine (DA) within the corpus striatum mediates turning behaviour in the rat^{1,2}. The turning occurs in a direction away from the striatum with the greatest activation of DA neurones. Contraversive turning also occurs after unilateral microinjection of agonists of γ -aminobutyric acid (GABA) into the substantia nigra (SN)^{3–6}, the site of origin of the dopaminergic nigrostriatal pathway. This finding was unexpected, as GABA inhibits neurones^{7,8} thought to be DA-containing cells^{9,10} in the SN. Hence, a GABA agonist would be expected to cause a decrease in the rate of release of striatal DA on the microinjected side and result in ipsiversive turning. Therefore, it is unclear whether a GABA agonist applied to the SN alters DA release in the ipsilateral striatum to evoke turning, or whether the turning results from a non-dopaminergic mechanism⁵. The dopamine antagonist, haloperidol, has been reported either to reduce significantly^{3,6} or exert no action on^{4,5} the contraversive turning response evoked by intranigral injection of a GABA-like compound. To clarify the action of GABA on the nigrostriatal dopaminergic system, we examined the effect of muscimol, a potent GABA agonist¹¹, on striatal DA metabolism and release. We report here that intraperitoneally (i.p.) administered muscimol increases the concentration of striatal homovanillic acid (HVA) and that intranigral perfusion of muscimol simultaneously evokes both contraversive turning and DA release from the ipsilateral striatum.

The effect of peripherally administered muscimol on the striatal levels of DA and its major metabolite, HVA, are given in Table 1. The concentration of striatal HVA was increased significantly at 0.5, 1 and 2 h after muscimol (2 mg per kg, i.p.) injection, whereas the concentration of DA was not altered. These results agree with those obtained in mice¹² and suggest that systemically administered muscimol can stimulate turnover or release of DA in the striatum.

Table 1 Effect of muscimol on rat striatal dopamine and homovanillic acid

Time (h) after muscimol	Concentration (nmol per g + s.d.)	
	Dopamine	Homovanillic acid
0	21.5 ± 3.3	1.9 ± 0.5
0.5	21.2 ± 7.8	2.8 ± 0.6*
1.0	24.3 ± 6.3	3.5 ± 1.1*
2.0	22.2 ± 7.9	4.0 ± 1.6*
4.0	20.9 ± 4.9	2.3 ± 0.9

Male Sprague-Dawley rats (Charles River, $n = 6$ per group) were given muscimol (2 mg per kg) intraperitoneally. Zero time control rats were treated with the vehicle (0.9% saline). Dopamine and homovanillic acid were extracted simultaneously from the same tissue sample and measured by a fluorometric method as described previously²⁵.

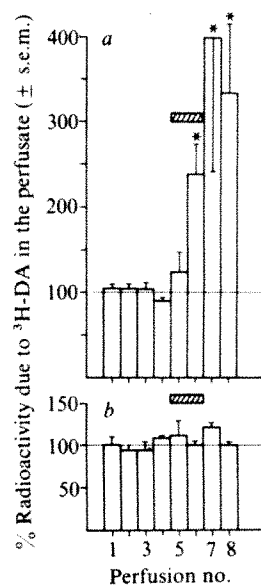
* Different from vehicle-treated control, $P < 0.02$ (Student's t test).

To evaluate the effect of muscimol on DA neurones further, the striatal release of the neurotransmitter was studied, using a push-pull cannula, while simultaneously perfusing a solution of muscimol through a second push-pull cannula in the ipsilateral SN. For push-pull perfusion, seven male Sprague-Dawley rats weighing 480–550 g, with a guide cannula chronically implanted in the corpus striatum and SN, were used. One week after surgery, a concentric push-pull cannula¹³ was lowered into the striatum and an artificial cerebrospinal fluid (CSF)¹⁴ containing 50 μ Ci ml⁻¹ of ³H-tyrosine (40–50 μ Ci mmol⁻¹) was perfused at a rate of 30 μ l min⁻¹. The experiment, modified from that of Nieoullon *et al.*¹⁵, consisted of eight serial 15-min perfusions of the striatum. During the fifth and sixth striatal perfusion, 10⁻⁴ M muscimol or the CSF vehicle was perfused simultaneously through the SN. A 400- μ l aliquot of each striatal perfusate was assayed for ³H-DA content using the ion-exchange method of Neff *et al.*¹⁶.

Each animal was used in two experiments spaced by a one-week interval. CSF was perfused through the SN in one and muscimol (10⁻⁴ M) in the other. Muscimol was used first in four

Fig. 1 The mean ($\pm$ s.e.m.) ³H-DA efflux detected in eight consecutive push-pull perfusates collected in the striatum. The radioactivity due to ³H-DA is expressed as a % of the spontaneous release value (indicated by the dotted line) as defined in the text. The horizontal bars (▨) indicate the periods during which either muscimol (10⁻⁴ M) (a) or CSF (b) was simultaneously perfused through the SN.

* Significant difference between groups ($n = 5$) using the Mann-Whitney U -test ($P < 0.002$).



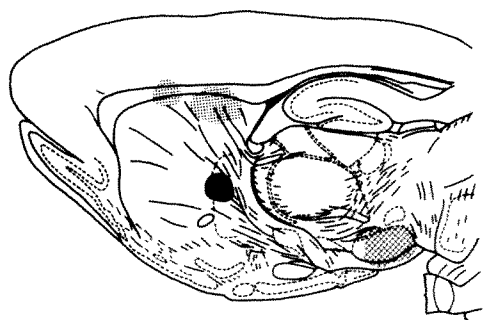


Fig. 2 A sagittal section of the rat brain depicting the areas perfused. The stippled section in the dorsal striatum encompasses the sites at which ^3H -tyrosine was perfused and ^3H -DA release was measured ($n=6$). The cross-hatched section in the SN encompasses the area in which muscimol was perfused ($n=7$), whereas the site in the globus pallidus at which no increased ^3H -DA release was noted during the muscimol perfusion is indicated by the black spot.

animals. The mean amount of ^3H -DA in the four perfusates preceding the SN perfusion was used as a value indicative of spontaneous ^3H -DA release (SR). ^3H -DA in each perfusate was expressed as a percentage of the SR and compared between muscimol and CSF perfusions using the Mann-Whitney U -test. The mean SR for 56 perfusates was 3.35 ± 0.62 (s.e.m.) nCi per ml of effluent. Each perfusion site was verified using the atlas of König and Klippel¹⁷.

In five rats, both contraversive turning and a significant efflux of ^3H -DA within the ipsilateral striatum were observed. As shown in Fig. 1, the efflux of ^3H -DA rose significantly during the muscimol perfusion and remained elevated in the subsequent perfusates. ^3H -DA release reached a maximum of 400% of SR in the first striatal effluent collected after stopping muscimol perfusion. The contraversive turning response to muscimol began with latencies varying between 30 s and 8 min, possibly due to slight differences in cannula placement. As with the release of ^3H -DA, contraversive turning and postural asymmetries also persisted after the cessation of the muscimol perfusions. Perhaps the persistent ^3H -DA efflux, circling behaviour, and contralateral postural asymmetries were caused by muscimol which had diffused into surrounding tissue. If as little as 0.01% of the perfused muscimol solution had diffused into the SN, it would be equivalent to a 1-ng microinjection of muscimol, a dose which has been reported to evoke contraversive turning⁵.

Increase of ^3H -DA release occurred only during or following the perfusion of muscimol which concomitantly evoked contralateral turning. Hence, only animals which both turned contraversively in response to muscimol and had verified neo-striatal and nigral perfusion sites were included in the group data. In one animal which did not turn during the muscimol perfusion, there was no increase in ^3H -DA in the striatal perfusate. As shown in Fig. 2, one striatal perfusion site was actually in the globus pallidus. Although this animal turned during the muscimol perfusion, no significant ^3H -DA efflux was observed from the pallidal site.

The SN perfusion of the potent GABA agonist, muscimol, both releases ^3H -DA in the ipsilateral striatum and evokes contraversive turning, supporting the hypothesis that muscimol-evoked turning and striatal DA release are causally related and suggesting that striatal DA mediates the turning. Muscimol may act to release DA neurones from tonic inhibition mediated by other SN neurotransmitters which include: substance P (ref. 18), acetylcholine¹⁹, serotonin¹⁹, dendritic DA²⁰ and glycine²¹. Using the 'encephalé isolé' preparation in the cat, Chéramy *et al.*²²⁻²⁴ have already demonstrated that several of these putative transmitters can modulate striatal ^3H -DA release when they are perfused through the SN. Future studies of the effects of

muscimol on these substances may help to determine the precise mechanism whereby intranigally administered muscimol evokes both contraversive turning and striatal DA release.

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Coupled motoneurones are part of the crayfish swimmeret central oscillator

SWIMMERETS in the crayfish are the paired ventral abdominal appendages which beat in a metachronal rhythm during behaviours such as swimming and burrow ventilation. Each swimmeret is driven by alternating bursts of impulses in antagonistic power- and return-stroke motoneurones. Although proprioceptive input affects the detailed structure of the rhythm, the basic motor programme can be elicited from the deafferented abdominal central nervous system (CNS) by tonic stimulation of single 'command fibres' in the ventral nerve cord¹⁻³. Each swimmeret is controlled by a hemiganglion capable of generating the rhythm in isolation, although normally the most posterior swimmeret ganglion (the 5th) acts as a pacemaker, regulating the activity of the other ganglia through a series of coordinating fibres located in the medial region of the ventral nerve cord⁴. I report here the preliminary results of an investigation into the structure of the central neural oscillator controlling swimmeret movements in crayfish. The results suggest that motoneurones in this system do not act merely as output elements which passively relay the activity of an oscillator formed from pre-motor neurones within the CNS, but that the motoneurones themselves are an integral part of the pattern generating circuits. There is both excitatory and inhibitory central coupling between motoneurones mediated either by electrical or graded chemical synapses. Furthermore, the activity of some motoneurones can directly influence the intensity, period, and phase of the oscillation.

Experiments were performed on the 4th abdominal ganglion of *Pacifastacus leniusculus*, which was attached to the rest of the

abdominal CNS, but isolated from the peripheral and rostral nervous system. Intracellular recordings were made with microelectrodes penetrating neuropilar regions of motoneurons from the dorsal surface of the desheathed ganglion. Extracellular pin electrodes were placed on the anterior and posterior branches of the 1st root, for recording and stimulating return-stroke and power-stroke motoneurons respectively. In some of the experiments reported here rhythmic motor output could be elicited by stimulating either the anterior or posterior branch of the 1st root at 25 Hz for 1–2 s. No attempt was made to stimulate command fibres. Occasionally, spontaneous rhythmic output was observed without any prior stimulation. The results of one typical experiment are described in detail below and then a summary is given of conclusions derived from a number of experiments.

A neurone was identified as a power-stroke motoneurone because it had an axon in the posterior branch of the 1st root, but not in the anterior branch (Fig. 1*a, b*). During spontaneous rhythmic output its membrane potential showed subthreshold oscillations, receiving excitatory postsynaptic potentials (e.p.s.ps) late in the burst of spikes recorded from other neurones in the posterior 1st root, and inhibitory post-synaptic potentials (i.p.s.ps) during the burst in the anterior 1st root (Fig. 1*c*). It was thus likely to be a fast excitor power-stroke motoneurone⁵. Injection of subthreshold depolarising current into this neurone when the rhythmic output was not being expressed caused an increase in the tonic spike frequency of other neurones with axons in the posterior 1st root. Conversely, hyperpolarising this neurone caused a decrease in the spontaneous spike frequency of such neurones (Fig. 2*a*). Since these effects result from subthreshold changes in membrane potential in a CNS isolated from the periphery, I conclude that this power-stroke excitor motoneurone is coupled to other power-stroke neurones either through nonrectifying electrical synapses or through graded chemical synapses in which there is a continuous release of excitatory transmitter by the resting neurone which can be increased by depolarisation and decreased by hyperpolarisation⁶.

This motoneurone was also coupled to pre-motor elements. When the motoneurone did not receive rhythmic input, it often received a tonic barrage of apparently unitary e.p.s.ps at ~20 Hz, which was continuous for many minutes with a very stable frequency. The frequency of this barrage was not affected by low levels of injected current (which were sufficient to

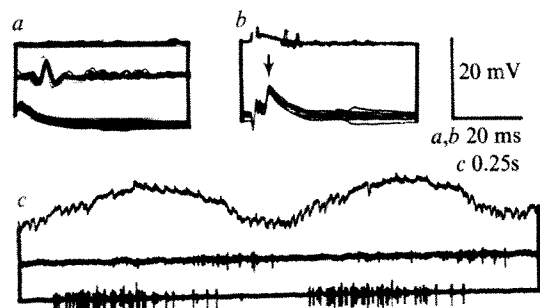


Fig. 1 Identification of a fast power-stroke excitor motoneurone. *a*, Triggering the oscilloscope sweep from a spike recorded intracellularly (lower trace, induced by injecting depolarising current) shows a phase-locked spike recorded extracellularly in the posterior (power-stroke) branch of the 1st root (middle trace) but none in the anterior (return-stroke) branch (upper trace). *b*, Stimulating the posterior branch causes an antidromic spike (arrow) which is recorded intracellularly. A spike is also recorded in the anterior branch, but not consistently. *c*, During periods of oscillator activity, the motoneurone (upper trace) receives rhythmic subthreshold depolarisations approximately in phase with bursts of spikes of power-stroke motoneurons in the posterior branch (lower trace) and in antiphase with the weak burst of spikes of return-stroke motoneurons in the anterior branch (middle trace).

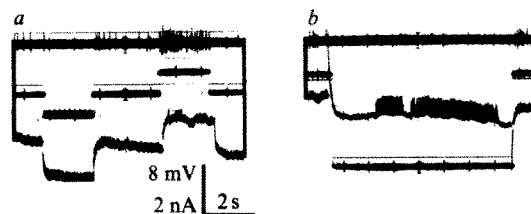


Fig. 2 *a*, Hyperpolarising current (middle trace) injected into the motoneurone (lower trace, bridge circuit only approximately balanced) decreases the spontaneous spike frequency of units in the posterior branch (upper line), while depolarising current increases their frequency. *b*, Hyperpolarising the motoneurone partially inhibits an excitatory presynaptic neurone.

demonstrate the motoneurone coupling), but with larger amplitudes of hyperpolarising current, the e.p.s.p. barrage became discontinuous (Fig. 2*b*). When present, the e.p.s.ps still occurred at ~20 Hz, but the previously continuous barrage was now broken by silent periods up to several seconds in duration. The e.p.s.ps were augmented in amplitude by the hyperpolarisation and thus were probably chemical. No spikes recorded in the anterior or posterior 1st root were phase locked to these e.p.s.ps, and so they are unlikely to be the result of reciprocal coupling from another motoneurone. Whatever the origin of these e.p.s.ps, the result demonstrates that this power-stroke excitor motoneurone is coupled to its own pre-motor excitor neurones, which suggests a positive feedback loop within the CNS.

When the CNS was producing the rhythm which in the intact animal causes swimmeret beating, the parameters of the oscillation could be altered by injecting current into this single motoneurone. Hyperpolarising current (~3 nA) caused a reduction in the number of units active during the power-stroke phase of the cycle and an increase in the activity of units in the return-stroke phase of the cycle. There was also a decrease in the frequency of oscillation and a disturbance of the strict reciprocity of power- and return-stroke activity (Fig. 3*a*). This could be seen not only in terms of the motor output as recorded extracellularly in the 1st root, but also as the synaptic input impinging upon the motoneurone. The i.p.s.ps during the return phase disappeared, probably because the membrane potential was near their reversal potential, while the e.p.s.p. barrage of the power-stroke phase became prolonged. These e.p.s.ps were similar in shape and intra-burst frequency to those described above in the quiescent preparation, but their identity remains a matter of speculation. More extreme hyperpolarisations (~5 nA) eliminated the rhythmic motor output altogether. Injection of small depolarising currents (1–2 nA) increased the number of units active in the power-stroke phase (including recruiting spikes in the injected motoneurone itself) and decreased the activity during the return-stroke phase. Hyperpolarising currents had the opposite effect (Fig. 3*b*). The depolarising current had no significant effect on the period of the rhythm, but the hyperpolarising current slowed it by 6% ($P < 0.025$). Continuous injection of larger depolarising currents strongly inhibited return-stroke activity, and increased the activity of a number of power-stroke neurones to the extent that it became difficult to distinguish any rhythmicity in the motor output. However, injection of pulses of such depolarising current had a strong resetting effect on the oscillator, including both the power- and return-stroke phases of the rhythm (Fig. 3*c*).

The levels of current used in these experiments were low (≤ 5 nA), and thus it is likely that the changes in motoneurone membrane potential occurring in the absence of experimental interference are effective in producing the central interactions. Much larger currents (10–20 nA) injected into extracellular space immediately after withdrawing the microelectrode from the neurone had no effect. The preparation remained healthy for several hours, and both the coupling to other motoneurons and the effects on the oscillation of injecting current into the

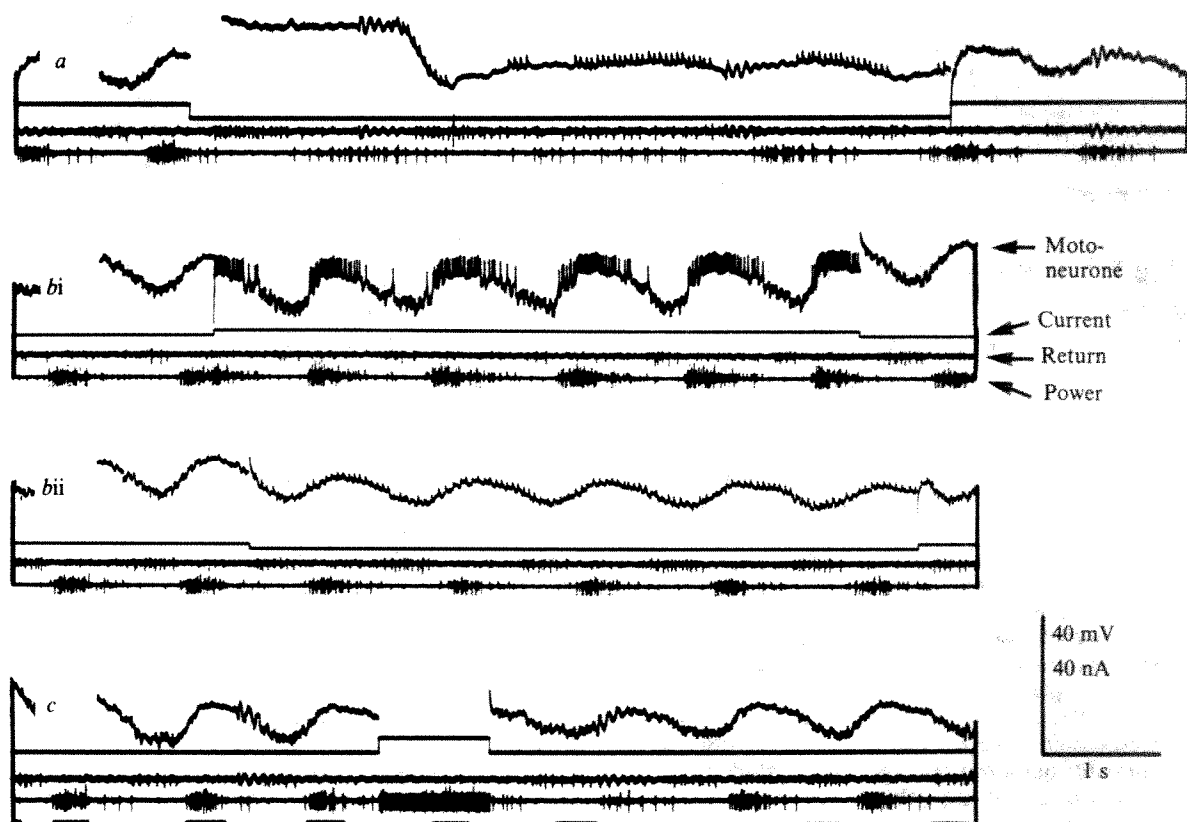


Fig. 3 *a*, Hyperpolarising current (2nd trace) injected into the motoneurone (upper trace) reduces the intensity of the power-stroke motor activity (bottom trace) and increases the period of the oscillation. There is greater than usual overlap between power- and return-stroke (3rd trace) activity. (The apparent motoneurone hyperpolarisation about 2 s after the start of current injection is caused by a change in the bridge balance.) *b*, Small depolarising currents (i) increase the intensity of power-stroke activity and decrease the intensity of return-stroke activity, while small hyperpolarising currents (ii) have the opposite effect. Note the slight difference in period between (i) and (ii). *c*, Injecting a pulse of depolarising current (bridge unbalanced) can reset the phase of the oscillator. The solid bars under the record indicate the times at which power-stroke activity would have been expected had there been no resetting.

motoneurone could be demonstrated repeatedly and consistently.

The oscillator has been reset by injecting current (<5 nA) into a single neurone in a total of four separate preparations—twice when depolarising current was injected into power-stroke motoneurons, and twice when depolarising current was injected into neurones which did not spike in response to depolarising current (up to 10 nA). Two other power-stroke motoneurons did not reset the oscillator when injected with similar levels of depolarising current. This variability may be due to differences in the sites of current injection relative to central output synapses of the motoneurons. Alternatively it may reflect genuine differences within the population of motoneurons. This latter is quite likely, since the lobster swimmeret motor pool consists of about 50 motoneurons innervating 11 muscles per swimmeret⁵, and the crayfish swimmeret system is probably just as complex. The gross distinction of power- and return-stroke motoneurons used in this work takes no account of basipodite rotators, exopodite openers, closers, benders and so on which cannot yet be identified in the isolated central nervous system. To determine what proportion of the total motor pool has access to the oscillator will require many more experiments.

Penetrations of power- and return-stroke motoneurons in more than 10 preparations which were not oscillating (and whose motoneurons could therefore not be tested for access to the oscillator) has revealed that coupling between motoneurons is widespread. Only rarely have motoneurons been found which did not exhibit some form of coupling. In general, this coupling is organised such that depolarising current

injected into one motoneuron excites its synergists and inhibits its antagonists, while hyperpolarising current has the opposite effect. In at least two pairs of power-stroke motoneurons the coupling is reciprocal and seems to be the result of an electrical synapse. Interactions are strongest between ipsilateral motoneurons within a ganglion, but have also been shown between motoneurons in adjacent ganglia. As yet no coupling has been found between contralateral motoneurons.

These results show that in the neural system controlling swimmeret movements the activity of at least some motoneurons can influence the intensity, frequency, and phasing of the rhythmic motor output from an isolated CNS. This suggests that these motoneurons themselves are part of, or at least can interact with, the oscillator circuit, and are thus not simply passive output elements. Furthermore, since none of the motoneurons from which I have recorded are endogenous pacemakers, the parameters of the oscillator must be a property of a network of neurones. The coupling between motoneurons demonstrated in the quiescent CNS may be a result of direct connections between the motoneurons, or may be mediated by interneurons, perhaps some of the same interneurons as those involved in the oscillator circuit.

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Evidence for the functional involvement of carotenoids in the photoperiodic reaction of spider mites

PHOTOPERIODISM in insects and mites depends on the presence of light-absorbing pigments, which are probably located in the central nervous system; photoperiodic responses are mediated by the direct action of light on these nervous elements¹. Until now, pigments involved in photoperiodic processes such as diapause and polymorphism have not been identified. Different groups of pigments have been named by various authors as possible candidates for this function. A biologically active role based mainly on action spectra studies and the absorption characteristics of isolated pigments has been suggested for pterins², carotenoids³, a combination of carotenoproteins, flavoproteins and haemoproteins⁴ and bile pigments⁵. Carotenoids, pterins and flavins have also been proposed as possible photoreceptor pigments in studies on the spectral sensitivity of circadian rhythms⁶. According to Lees¹, however, the usefulness of action spectra in identifying the light acceptor is limited, as spectra for photoperiodic action cannot at present be matched against absorption curves. A different approach was used by Zimmerman and Goldsmith⁷, who reared *Drosophila* on a carotenoid-free diet and showed that in one generation the sensitivity of the visual receptors was lowered by 3 log units because of lack of rhodopsin. At the same time the photosensitivity of the rhythm of pupal eclosion in the cultures was not affected, from which it was concluded that the pigment involved in mediating light effects on the circadian rhythm in *Drosophila* is not a carotenoid derivative. We demonstrate here that carotenoids are functionally involved in the photoperiodic induction of diapause in the spider mite *Tetranychus urticae* using a method involving pigment mutants.

In *T. urticae*, only adult females may undergo a facultative diapause, induced by short-day photoperiods experienced during the larval and nymphal stages. With the test regime used (10L:14D, 18 °C) the different wild-type strains showed a 100% diapause⁸. From a wild-type strain we have isolated seven different pigment mutants, all due to single recessive mutations which interfere with the uptake and oxidative metabolism of β -carotene in these mites⁹. It seemed that only the albino mutant showed a lowered diapause response, all other pigment mutants exhibiting 100% diapause in the above test regime. Four albino mutants, which arose spontaneously in different wild-type strains, all responded with a lowered diapause, although all four originated from wild-type strains which give 100% diapause in the test conditions. Complete diapause has never been observed in any albino strain; although highly variable in expression (diapause responses range over 0–80% in different tests), the percentage diapause in albino strains is always markedly reduced in comparison with wild type. Back-crossing of three of the albino mutants to wild type for 10 generations did not restore the capability to diapause. As crossing-over is thought to occur very frequently in these mites¹⁰, these findings are a strong indication for a functional involvement of the albino locus in the photoperiodic reaction of *T. urticae*.

Even stronger evidence for a functional connection between the presence of carotenoid pigments and diapause was obtained in other crossing experiments between wild type and albino. For these crosses use was made of the male haploidy (arrhenotoky)

in *T. urticae*. The out-cross of the albino mutant to wild type gives hybrid daughters which are phenotypically wild type and diapause normally. Crossing these hybrid females to albino males produces a mixed female offspring, consisting of wild type and albino in a 1:1 ratio. Surprisingly, both these categories show a 100% diapause when tested in the photoperiodic regime mentioned above. Albino females of this offspring, after random mating with their brothers, which also consisted of a 1:1 ratio of wild type and albino, again gave rise to a mixed offspring of wild-type (hybrid) and albino females. From these, the wild-type females all diapaused on short-day treatment, whereas the albino daughters showed the same lowered diapause response as described for the different albino strains. From these crosses it seems that 100% diapause in albino is realised only when the albino daughters come from hybrid, phenotypically wild-type mothers. Apparently a maternal effect is responsible for the complete induction of diapause in albino females.

The lowered diapause response encountered in all albino strains and the fact that the capability to diapause could not be restored by a series of back-crosses to wild type, suggest that the albino locus exerts a pleiotropic effect, influencing both pigmentation and diapause induction. The discovery of the maternal effect in the crossing experiments described above confirms this supposition and leads us to conclude that carotenoids are functionally involved in diapause induction in *T. urticae*. With respect to pigmentation, a maternal effect, consisting of small red eyespots in albino males produced by wild-type hybrid females, has been demonstrated previously in these mites¹¹. This showed that the egg of spider mites transmits a certain amount of carotenoids, which may be used for the formation of eyespots. From the experiments described above it could be concluded that these maternally derived carotenoids may also be utilised by the embryo in the formation of a photopigment concerned in the induction of diapause. Very small amounts of pigment would probably suffice for this function. A more elaborate account of these and other results will be published elsewhere.

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Evidence for passive electrotonic interactions in red rods of toad retina

SINCE Baylor, Fuortes and O'Bryan¹ first demonstrated interactions between vertebrate photoreceptor cells, many types of inter-receptor influences have been described^{2–10}. While some of these interactions are mediated by horizontal cells^{1,2,6}, others apparently result from direct contact between the photoreceptors themselves^{1,3,4,7–10}. Fain *et al.*¹⁰ reported that the red rods of the toad *Bufo marinus* were coupled by gap (electronic) junctions, and that single rods received detectable signals from other receptors over a retinal area of about 0.5 mm². This

receptive field area is 4–100 times larger than those measured for photoreceptors of a variety of species^{1,4,7,9,11,12,15}. Fain *et al.* found this extensive spread of rod signals to be inconsistent with network models containing only passive elements and this raised the possibility that some active process, perhaps in the rod membrane, contributed to the spread of rod signals. We report here results which indicate that single rods sum signals over only 1/16th the area previously estimated; this smaller receptive field area implies more intrinsic noise, a larger response to single photon absorptions, and a larger signal-to-noise ratio than predicted by the previously reported results¹⁰. Furthermore we find that, as in other species (ref. 13, and Copenhagen and Owen, personal communication), the spread of low-amplitude, light-evoked responses between photoreceptors can be accounted for by purely passive electrical properties.

Bufo marinus red rods were impaled in eye cup preparations and identified by their characteristic slow waveforms^{3,14,15}, spectral sensitivities^{14–17}, and by injection of Procion yellow. Receptive field properties were determined using two different stimulus configurations.

Two of us (H.F.L. and D.R.C.) recorded rod responses to a long, narrow bar of light flashed at various regions of each rod's receptive field. This bar was moved across the retina in a stepwise fashion, perpendicular to the long axis of the stimulus, and at positions separated by either 9.0 or 13.5 μm the stimulus was flashed from three to six times. Multiple responses, recorded to flashes at each position, were computer averaged and the peak amplitudes plotted semi-logarithmically as a function of slit displacement (Fig. 1). For all cells tested, the light response decreased exponentially as the bar was moved from the centre to the periphery of the receptive field. Such behaviour is predicted by two-dimensional cable models and permits the spatial properties of the response to be characterised by a length constant λ (refs 18, 19). For 11 rods tested, the mean length constant was $24 \pm 5 \mu\text{m}$ ($\pm$ s.d.) (Table 1).

In independent experiments, receptive field properties of toad rods were measured by one of us (R.A.N.) using flashed circular spots of light of increasing diameter. A small, dim spot of light (13–30 μm diameter) was centred over the most sensitive region of the rod's receptive field, and photoreponses were recorded as the diameter of the spot was increased from 13 to 265 μm at a constant, dim light intensity (Fig. 2, inset). For the cell shown in Fig. 2, the response to these flashes increased asymptotically to a diameter exceeding 200 μm . The diameter of the summation area (95% V_{FF}) was taken as 160 μm . The continuous curve represents the relationship between spot diameter and central voltage response for a continuous two-dimensional resistive sheet with a length constant of 18 μm . This relationship is described by

$$V(d) = V_{\text{FF}} \left\{ 1 - \left[\frac{d}{2\lambda} K_1 \left(\frac{d}{2\lambda} \right) \right] \right\}$$

where $V(d)$ is the voltage at the centre of the illuminated circle of diameter d , V_{FF} is the voltage with full field illumination, and K_1 is a modified Bessel function^{18,19}. For 14 rods tested in this manner, the mean length constant was $21 \pm 6 \mu\text{m}$ ($\pm$ s.d.).

These results indicate that red rods are functionally coupled over an area of approximately 0.03 mm^2 rather than 0.5 mm^2 as previously estimated¹⁰. Thus, each rod is functionally coupled to a maximum of 500–550 other rods rather than to 8,000–9,000 (refs 10, 20). We have also learned that two other investigators (ref. 21, and G. Gold, personal communication), recording from red rods in isolated *Bufo* retinal preparations (rather than in eye cups), have found summation areas comparable to those reported here.

This smaller receptive field size has several implications for the physiology of *Bufo* red rods. For example, the magnitude (r.m.s. value) of the intrinsic noise (membrane events not caused by the absorption of a photon) in a coupled rod should be inversely related to the diameter of the summation area (proportional to λ); this assumes that coupling serves to average

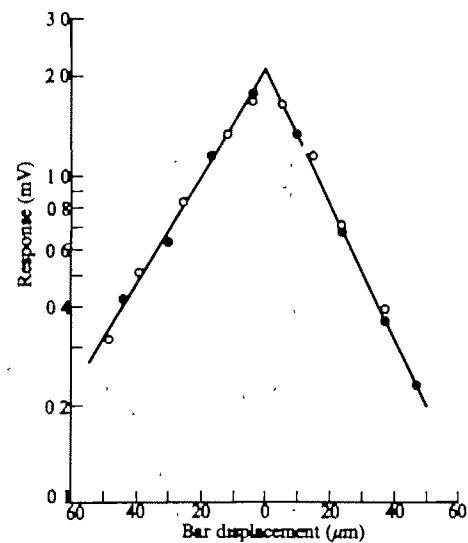


Fig. 1 Response of a *Bufo marinus* red rod as a function of the displacement of a narrow stimulus bar. The bar was moved through the receptive field of each cell twice. $\circ$, Data collected during the first pass of the bar; $\bullet$, data collected as the bar was moved in the opposite direction. Points are the computer average of three to six responses at each position. Solid lines were fitted by the method of least-squares error. The bar was produced by passing the light beam through a rectangular slit formed by two razor blades. The image of the slit on the retina had a nominal halfband width of 8 μm as determined by passing the image across a 5- μm entrance aperture to a photomultiplier tube which was in the position normally occupied by the eye cup. The length of the bar was limited by an iris diaphragm and was 750 μm in the central position. Responses were elicited by 40-ms flashes of 514 nm light delivering to the retina about 120 photons per μm^2 per flash. Details of the optical system and its calibration are described elsewhere⁷.

noise sources across many rods as it does with turtle cones¹³. Thus, the smaller summation area would increase estimates of intrinsic noise by a factor of ~ 4 . Similarly, estimates of the coupling resistance between *Bufo* red rods would be larger for this smaller summation area, and one would expect less shunting of signals to adjacent rods. Quantitatively, estimates of input resistance, and hence the signal generated by the absorption of a single photon, would be increased by a factor of ~ 10 . Therefore,

Table 1 Summary of length constants for 11 *Bufo marinus* red rods

Rod no.	λ_-	r_-^2	λ_+	r_+^2	V_{centre} (mV)
1	25.9	0.99	24.6	0.97	2.2
2	26.1	0.96	24.6	0.97	3.4
3	33.8	0.99	18.9	0.98	2.7
4	24.2	0.98	23.2	0.98	2.2
5	22.3	0.94	17.8	0.98	2.0
6	24.0	0.99	25.4	0.97	2.4
7	21.7	0.98	24.7	0.96	1.0
8	31.3	0.99	28.8	0.95	2.3
9	26.3	0.99	21.7	0.99	2.7
10	28.3	0.99	25.0	0.97	2.2
11	15.5	0.99	12.6	0.99	1.8

λ_- and λ_+ represent length constants on opposite sides of the centre for individual rods; r_-^2 and r_+^2 are the coefficients of correlation between the data and a simple exponential function (Fig. 1). V_{centre} is the amplitude of the light response when the stimulus bar was over the central (most sensitive) part of the field. Differences between λ_- and λ_+ could not be attributed to retinal position or optical factors. Similar asymmetries have been seen in other species (ref. 18, and Copenhagen and Owen personal communication). The mean of these 22 length constants is $23.9 \pm 4.7 \mu\text{m}$ ($\pm$ s.d.).

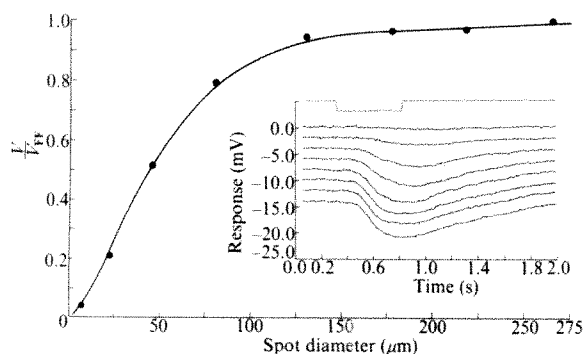


Fig. 2 Amplitude of photoresponses (circles) to 500-ms flashes of a dim circular spot of white light as its diameter was increased from 13 to 265 μm . The solid curve is the relationship between spot diameter and response amplitude for a continuous two-dimensional sheet which exhibits a length constant of 18 μm . Inset: photoresponses recorded (from top to bottom, respectively) at 13, 23, 47, 82, 131, 167, 219 and 265 μm spot diameters (curves displaced for illustration). Spots were generated with either an iris diaphragm (for the large spots) or with a plate containing a variety of apertures (for small spots). Spot diameters were calibrated by observing the image cast on the retina with a microscope containing an ocular reticle. Details of the optical system and its calibration are described elsewhere¹⁵.

the predicted signal-to-noise ratio for single photon capture would be increased by a factor of ~ 2.5 . Most importantly, our data show that the spatial properties of *Bufo* red rod responses are well described by an exponential for thin bar-shaped stimuli and a modified Bessel function for circular stimuli. Therefore, our results indicate that a network containing purely passive membrane and coupling resistances can account for the extent of spread of signals between *Bufo* red rods.

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Note added in proof: Detwiler *et al.*²² recently reported that the network of turtle rods possesses some properties of a high-pass filter. In the analysis of our data we have assumed a purely resistive network in modelling the spread of *Bufo* rod signals. It can be shown, however, that under the passive network assumptions used by Detwiler *et al.* (their Fig. 1b), the d.c. space constant is still described solely by the resistive network elements if determined from measurements of the peak response amplitude (where $dv/dt = 0$).

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Increase in free Ca^{2+} in muscle after exposure to CO_2

IT IS now established that a change in intracellular free Ca^{2+} forms a vital link in the action of certain hormones and neurotransmitters as well as in excitation–secretion and excitation–contraction coupling^{1–5}. Here we report an increase in intracellular free Ca^{2+} (Ca_i^{2+}), brought about by exposure of muscle fibres to CO_2 . This effect may provide an important clue to the mode of action of CO_2 in muscle cells as well as to a possible regulation of Ca_i^{2+} by changes in intracellular pH (pH_i).

Single muscle fibres from the barnacle *Balanus nubilus* were axially injected with the calcium-sensitive photoprotein aequorin⁶, prepared by chromatography from extracts of *Aequorea forskalea*⁴. Usually a period of 2 h was allowed for uniform distribution of the photoprotein internally, after which the light was recorded with an EMI photomultiplier tube (9634A), run at -900 V, with the output (time constant 10 ms) displayed on a pen recorder. The fibres were bathed in artificial seawater (ASW), composition (mM) NaCl 510, KCl 12.9, CaCl_2 11.8, MgCl_2 23.6, NaHCO_3 2.6 and TES 2.0 at pH 7.1 and temperature 17 – 23°C .

If the ASW was replaced with ASW which had been equilibrated with either 5, 10 or 100% CO_2 so that the values of the saline pH were 6.4, 6.1 and 5.0 respectively, the aequorin light emission increased over a period of 10–20 min, reaching in 100% CO_2 a new steady level which was 5–25 times (nine fibres) the initial resting value (Fig. 1b–d). This response to CO_2 was observed immediately after aequorin injection, although the rate at which the steady level was achieved was slower than after the equilibration period of 2 h. If the pH of 100% CO_2 –ASW was adjusted to 6.9 by addition of 100 mM NaHCO_3 (replacing NaCl) there was still a marked increase in aequorin light emission. Application of an ASW at pH 5.0 (buffered with 10 mM NaH_2PO_4 or 10 mM Tris-maleate) produced relatively little change (Fig. 1a) in light emission over 20 min (in some fibres a small increase and in others a decrease in light was observed, the maximum change being 0.3 times the resting light). These last two observations suggested that it was either a direct action of CO_2 or a change in pH_i that initiated the aequorin light response.

The fact that CO_2 does readily change pH_i in these and other cells is well documented^{7–10}. Application of 100% CO_2 –ASW produces a change in pH_i of about 1 unit from 7.2 to 6.2 in crustacean muscle fibres after a period of 10–15 min^{7,8}, whereas phosphate–ASW (pH 5.0) produces a change in pH_i of only about 0.2 pH units over the same period⁷. We have also determined that the increase in light emission seen in CO_2 –ASW was not brought about simply by a direct action of either H^+ or CO_2 on the apparent affinity of aequorin for Ca^{2+} . First, in a synthetic internal solution having the following composition (mM) KCl 150, NaCl 20 and MgCl_2 5, with added Ca^{2+} about 60×10^{-6} , at pH 7.1 with K_2CO_3 (Johnson Matthey) there was no increase in the aequorin light emission after equilibration with 100% CO_2 (pH 5.0 finally) and the records in fact indicate a small inhibitory effect which would be expected from the known values of the apparent binding constants of these ions to the photoprotein at

these different pH values¹¹ (Fig. 1e–g). *In vivo*, the pH_i change would be less than occurred in this assay (see above). Second, the increase in light emission was also accompanied by an increase in isometric tension development (Fig. 1h), which was equivalent to about 0.6% of the maximum tension, P_0 , for this preparation (49 N cm⁻²)¹⁴.

All these experimental observations strongly suggest that the increase in aequorin light emission in CO₂-ASW does in fact represent an increase in free Ca²⁺. Assuming that the free Ca²⁺ is proportional to (aequorin light)^{0.4} (ref. 11), and that the change in Ca²⁺ is uniform within the fibre at the peak of the response, the increase in free Ca²⁺ by 100% CO₂ is calculated to be approximately two to four times, increasing from 70–100 nM (refs 12 and 13) to 140–400 nM, respectively.

It was important to determine whether this increase in free Ca²⁺ resulted from either a stimulation of calcium influx across the surface membrane or a release from internal stores or a combination of both processes. Replacement of ASW with OCa-ASW (Na replaced) reduced the aequorin light response in 100% CO₂ to 7–27% ($n = 6$) of the control response in 100% CO₂-ASW, after 12–50 min in OCa-ASW (Fig. 2) and the

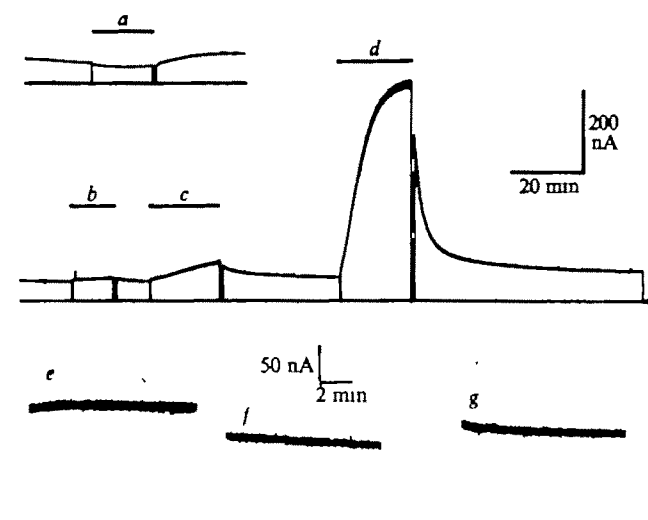


Fig. 1 a–d, Effects of extracellular pH and CO₂ on aequorin light emission from a single muscle fibre in ASW at pH 7.1 TES (*N*-Tris(hydroxymethyl)methyl-2-aminoethanesulphonic acid) a, ASW at pH 5.1 (10 mM Na₂HPO₄), b, c and d, ASW equilibrated with 5, 10 and 100% CO₂ at final pH 6.4, 6.1 and 5.0, respectively. Baseline is zero light emission. Ambient light 5 nA. Fibre membrane potential (E_m) –51 mV. e–g, Effects of pH and CO₂ on aequorin light emission from a simulated muscle solution (see text). e and g, controls at pH 7.1; f, after equilibration with 100% CO₂ at final pH 4.8. Each trace shows a separate assay, and starts 3 min after 0.3 μ l saturated aequorin solution was injected into each 50- μ l aliquot and rapidly mixed. Ambient light 2 nA. h, Isometric tension (i) and aequorin light emission (ii) from a single muscle fibre, showing response to 100% CO₂-ASW at pH 5.0. Tension was recorded with an RCA mechanoelectric force transducer connected to a bridge circuit and Devices pen recorder⁴. Fibre diameter 1,150 μ m, E_m –48 mV. Ambient light 5 nA. Calibration for tension 1 mN = 0.2% P_0 .

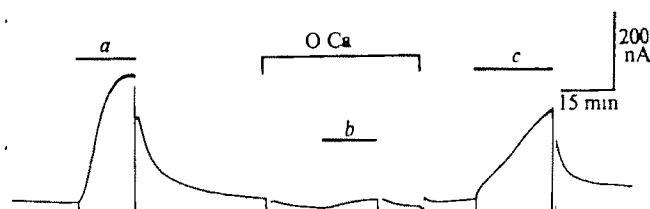


Fig. 2 Effects of OCa (Na substituted) saline on the response to CO₂ of aequorin light emission from a single muscle fibre (E_m –51 mV). a and c, 100% CO₂-ASW; b, 100% CO₂-OCa; final pH both 5.0. Ambient light 5 nA. Note the reduction in resting light emission in OCa-ASW (see ref. 13).

tension response was also abolished. This result seemed to support the idea that a stimulation of Ca²⁺ influx was involved in the response to CO₂. However, measurement of the resting ⁴⁵Ca²⁺ influx across the muscle membrane using the intracellular scintillator probe^{14,15} showed that the unidirectional Ca²⁺ influx was reduced in 100% CO₂-ASW by 40% over a 20-min period (three fibres) (Fig. 3a), whereas in phosphate-ASW (pH 5.0) there was no effect over a similar time (three fibres). These fibres had first been injected with 1 M K₂EGTA solution giving a final intracellular concentration of 11–18 mM per kg in order for the probe to assess accurately the intracellular ⁴⁵Ca²⁺ concentration.

The Ca²⁺ efflux, measured after axial injection of ⁴⁵Ca²⁺ (ref. 16), indicated that in 100% CO₂-ASW there was an average reduction of 53% (three fibres) (Fig. 3b), and in phosphate-ASW (pH 5.0) there was an average reduction of 36% (three fibres) in the rate of Ca²⁺ loss from the cell. Thus, if the Ca²⁺ influx and the Ca²⁺ efflux are approximately equal, then the effects of 100% CO₂ would be to produce a net Ca²⁺ influx equivalent to 13% (about 0.1 pmol cm⁻² s⁻¹) of the resting unidirectional influx. The failure of ASW-phosphate (pH 5.0), which would produce a larger net influx equivalent to 36% of the resting unidirectional influx, to increase the free Ca²⁺ in a similar way to the effect of CO₂-ASW argues against the smaller net influx of Ca²⁺ observed in CO₂-ASW being by itself responsible for the increase in free Ca²⁺. Also, external La³⁺ (2 mM), known to inhibit both the resting Ca²⁺ influx and efflux^{14,17,21} as well as the increase in free Ca²⁺ which occurs in Na-free ASW in these fibres¹⁷, failed to affect significantly the CO₂-induced increase in free Ca²⁺ (the mean response to CO₂ in the presence of La³⁺ was 93% of the control response in ASW ($n = 3$)).

It seemed possible that changes in membrane potential, which have been reported to occur in muscle fibres exposed to CO₂-ASW^{8,18} could have been releasing Ca²⁺ from internal Ca²⁺ stores within the sarcoplasmic reticulum (SR). Measurements with an axial, intracellular electrode (100 μ m tip) filled with KCl (3 M) showed that 100% CO₂-ASW produced a slow depolarisation of 10–15 mV over 20 min (seven fibres). Depolarisations of similar amplitude and duration, produced in the same fibres by 60 K⁺-ASW, resulted in either transient increases in light emission which were less than 10% of the increases observed with 100% CO₂-ASW, or no increases at all.

In conclusion, it seems that external CO₂ application results in an increase in free Ca²⁺ in these muscle fibres and that this cannot readily be attributed to either a net Ca²⁺ influx alone or to a depolarisation of the surface membrane, although a CO₂-induced depolarisation may contribute up to 10% of the observed increase in aequorin light emission. The requirement of the response to CO₂ for extracellular Ca²⁺, as shown by the effects of Ca²⁺-free saline, means that the small net Ca²⁺ influx induced by CO₂-ASW should not be ruled out entirely as a possible cause of the response. If, for example, the CO₂-induced reduction in pH_i also impairs the functioning of the Ca²⁺-ATPase pump in the membrane of the SR, then a small net influx of Ca might lead to an increase in free Ca²⁺. There is evidence from frog skinned muscle fibres that the Ca²⁺ loading of the SR at physiological free Ca²⁺ levels is decreased by a reduction of pH from 7.0 to 6.2 (ref. 19).

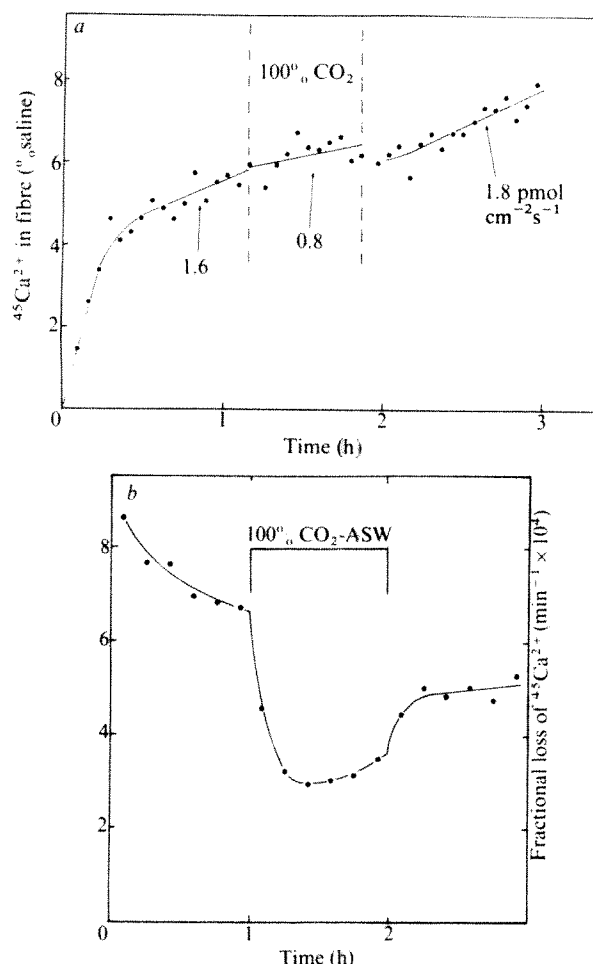


Fig. 3 *a*, Effect of CO_2 on $^{45}\text{Ca}^{2+}$ influx, measured in a single muscle fibre using an intracellular scintillator probe. $^{45}\text{Ca}^{2+}$ concentration in muscle fibre (as percentage extracellular $^{45}\text{Ca}^{2+}$ concentration) is plotted against time of immersion in $^{45}\text{Ca}^{2+}$ saline (0.1 mCi ml^{-1}): ASW ($\text{pH } 7.1$), 100% CO_2 -ASW ($\text{pH } 5.0$). Values of the influx ($\text{pmol cm}^{-2} \text{s}^{-1}$), calculated from the slope of the line drawn by linear regression analysis, are shown. Initial fast phase represents filling of extracellular 'cleft' system^{14,15}. Intracellular EGTA 14 mM per kg, $E_m = -47 \text{ mV}$; diameter $1.240 \mu\text{m}$. *b*, Effect of CO_2 on residual $^{45}\text{Ca}^{2+}$ efflux from single muscle fibre which had been axially injected with $^{45}\text{CaCl}_2 + \text{TES}$ ($\text{pH } 7.1$). $^{45}\text{Ca}^{2+}$ appearing in external ASW was counted by liquid scintillation¹⁶. Fractional loss of $^{45}\text{Ca}^{2+}$ ($\text{min}^{-1} \times 10^4$) plotted against time after injection. The pH of 100% CO_2 -ASW was 5.0. $E_m = -48 \text{ mV}$.

A second possible mechanism for the CO_2 response is a stimulation by the reduction in pH_i of a Ca^{2+} -dependent release of Ca^{2+} from the SR, similar to the type which has been proposed to occur in the normal E-C coupling in skeletal muscle fibres²⁰. Relevant to this is the finding that exposure of barnacle muscle fibres to OCa-ASW results in a complete loss of the electrically evoked calcium transient and tension response within 10–15 min (C. C. A. and R. Burles, unpublished). This is thought to reflect a requirement of the E-C coupling process itself for extracellular Ca^{2+} , as the caffeine-releasable internal stores of Ca^{2+} are still reasonably intact at this time²¹ and the amplitude of the extra Ca^{2+} influx which occurs during electrical stimulation in normal saline is insufficient to account for the observed tension development^{14,15}, even if one assumes that all the extra Ca^{2+} binds directly to the contractile proteins.

A third possibility is that there is displacement of Ca^{2+} by H^+ either from some organelle other than the SR or from Ca^{2+} -binding proteins in the sarcoplasm. In this case the effect of OCa-ASW on the CO_2 response could be explained by a ready depletion of this labile intracellular Ca^{2+} store, following the

rapid decline in free Ca_i^{2+} seen in these circumstances (see Fig. 2).

Evidence demonstrating that it may be a reduction in pH_i and not the increase in intracellular CO_2 concentration which is the stimulus for the increase in free Ca_i^{2+} was obtained by injecting muscle fibres with various buffer solutions (1 M $\text{KH}_2\text{PO}_4/0.05 \text{ M Na}_2\text{HPO}_4$, 1 M HEPES or 1 M Tris-maleate). Solutions of $\text{pH } 5.0$ – 5.5 transiently increased the aequorin light emission by up to 10 times, whereas solutions at $\text{pH } 7.2$ did not.

Interestingly, aequorin light emission from squid giant axons is reduced by 5% CO_2 -ASW, and this has been attributed solely to changes in Ca^{2+} fluxes across the axon membrane^{22,23}. The opposite effect of this level of CO_2 on barnacle muscle may mean that in muscle, Ca^{2+} is being released from an intracellular source which is absent in nerve.

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Requirements for therapeutic inhibition of sickle haemoglobin gelation

KINETIC studies on haemoglobin S gelation have shown that following initiation of the reaction there is a pronounced delay before the appearance of the polymer^{1–5}. The delay time is extremely sensitive to physiological variables, such as total haemoglobin concentration and fractional saturation with ligand, and moderately sensitive to pH , salt concentration and temperature (near 37°C)^{1,5–10}. Changes in these variables which decrease the delay time seem to be associated with increased clinical severity of sickle cell disease^{1,11}. These findings have been explained by a pathophysiological mechanism in which a decrease in the delay time of intracellular gelation, relative to the capillary transit time, increases the probability of intracapillary sickling; blockage of the microcirculation is then possible, with consequent tissue anoxia and organ damage. An increase in the delay time, permitting cells to escape the narrow capillaries before gelation has begun, is therefore predicted to result in amelioration of the disease^{1,11}. We present here results of kinetic experiments designed to help answer the question: how much must the delay time be increased to obtain a given therapeutic effect in patients?

The gelation kinetics of haemoglobin S were investigated in mixtures with normal adult haemoglobin (A) or fetal haemoglobin (F). Increased proportions of these normal haemoglobins, if homogeneously distributed in the red cell population, are known to be associated with decreased clinical severity¹²⁻¹⁶. By correlating the delay time data on specific haemoglobin mixtures with the data on the associated clinical course, we obtained a quantitative estimate of the increase in the *in vitro* delay time required to produce a particular clinical status. These results, together with other kinetic data, were then used to evaluate various therapeutic strategies.

The kinetic results reported here were derived from temperature-jump experiments in which the initiation of gelation was instantaneous relative to the delay time⁷. If initiation is not instantaneous, the observed delay will always be longer, this effect increasing with decreasing delay time. In such conditions, the increases in delay time will be smaller than those reported here. Zarkowsky and Hochmuth⁹, for example, found an increase of only 35-fold for the delay time of morphological sickling of A/S (heterozygous) trait cells relative to S/S (homozygous) cells. Kinetic studies on sickle cells by Rampling and Sirs²⁵ suggest that deoxygenation was not sufficiently rapid to resolve the delay time for S/S cells in the Zarkowsky and Hochmuth experiments. We point out, however, that deoxygenation *in vivo* may also not be fast relative to the delay time, so that experiments designed to mimic this situation will show a decreased sensitivity of the delay time to solution variables.

Figure 1 shows the delay time for the thermally induced gelation of mixtures. The effect of deoxyhaemoglobin F on the delay time is considerably larger than the effect of deoxyhaemoglobin A. This result is expected from previous equilibrium and minimum gelling concentration data, which showed that deoxyhaemoglobin F has a larger inhibitory effect than deoxyhaemoglobin A^{6,7,21-24}. A detailed experimental and theoretical analysis of both the kinetic and equilibrium data will be presented elsewhere²⁰.

In the double heterozygous conditions of sickle/ β^+ -thalassaemia, red cells contain about 15–30% haemoglobin A¹². In mixtures of these compositions we measured a 10–100-fold increase in delay time. Although, as in homozygous sickle cell disease, the clinical severity is quite variable, S/ β^+ -thalassaemia is in general less severe than S/S disease^{12,13}. Our kinetic results suggest, then, that some therapeutic effect might result if the delay time of gelation were increased by a factor of 10–100. In the double heterozygous condition of sickle/hereditary persistence of fetal haemoglobin (S/HPFH), red cells contain about 20–30% haemoglobin F^{12,14}. Such mixtures increase the delay time 10^3 – 10^4 -fold. Since this condition produces a much milder clinical course than S/S disease^{12,14}, increasing the delay time by a factor of 10^3 – 10^4 can reasonably be expected to have a major therapeutic effect in patients. Finally, in sickle cell trait, red cells contain ~60% haemoglobin A and 40% haemoglobin S^{13,15}. The increase in delay time found for a mixture of this composition is ~ 10^6 -fold. Patients with sickle cell trait rarely show any clinical manifestations¹⁶. Increasing the delay time, then, by about a factor of 10^6 , could be expected to produce a 'cure' of S/S disease.

Three different strategies to increase the delay time of intracellular gelation have been suggested. One is to directly inhibit polymerisation with molecules that competitively bind to sites of intermolecular contact. A second is to inhibit polymerisation indirectly by increasing the haemoglobin oxygen affinity, thereby increasing the fractional saturation in capillaries. A third strategy, suggested by previous kinetic studies, is to decrease the intracellular haemoglobin concentration^{1,11}.

We can estimate the requirements for each strategy. The decrease in intracellular haemoglobin concentration, Δc , required to produce a given increase in the delay time, t_d , can be directly calculated from the measured concentration dependence: $d \log t_d / d \log c = -(35-55)$ (refs 1, 6, 20). In the limiting case where a molecule binds to a single site and completely prevents incorporation into the polymer, the frac-

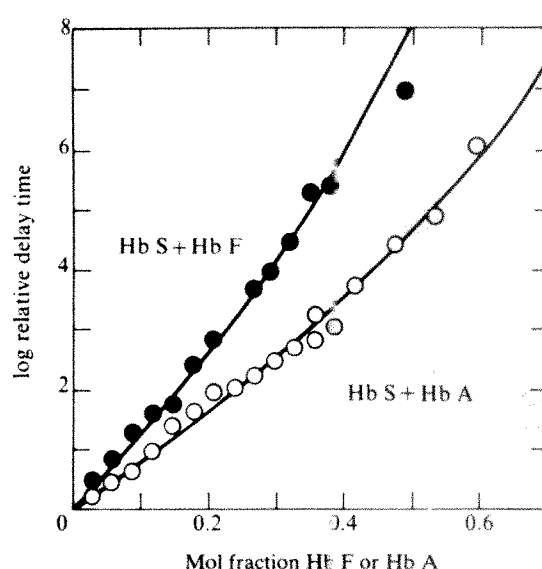


Fig. 1. Effect of deoxyhaemoglobin A and F on the delay time of thermally induced gelation. The relative delay time is defined as the ratio of the delay time for the mixture, t_d , to the delay time for deoxyhaemoglobin S alone, $t_d(S/S)$, at the same total haemoglobin concentration. To obtain relative delay times over seven decades at the same total haemoglobin concentration and temperature (286 g l^{-1} and 23.4°C for S-F mixtures, 270 g l^{-1} and 23.0°C for S-A mixtures), delay times were measured as a function of total haemoglobin concentration and temperature using a turbidity technique⁷ and then extrapolated to the same values. All mixtures were initially prepared in the oxy form to permit complete hybridisation¹⁷⁻¹⁹. The curve through the S-F mixture points is theoretical, and is obtained from the equation $\log(t_d/t_d(S/S)) = -s \log(1 - X_F)^2$, which assumes that the molecular species— $\alpha_2\beta_2^S$, $\alpha_2\gamma_2$ and $\alpha_2\gamma\beta^S$ hybrid—are present in a binomial distribution¹⁷⁻¹⁹, and that only the $\alpha_2\beta_2^S$ species polymerises. A least-squares fit to the data gives $s = 13.5$. Details of the experimental procedures and data analysis will be presented elsewhere²⁰.

tional saturation of the site with inhibitor, x , can be calculated from the relation: $d \log t_d / d \log(1 - x) \approx -10$. This derivative is an average of values obtained from experiments in solutions partially saturated with carbon monoxide and from the S-F mixture data in Fig. 1^{20,26}. It is smaller than the corresponding derivative for the concentration dependence because the inhibited molecule, while incapable of being incorporated into the polymer, still occupies space in the solution and thereby increases the kinetically effective concentration of the uninhibited, polymerising molecules^{20,26-29}. Oxygen affinity calculations are more speculative, as the only relevant kinetic result is $d \log t_d / dy \approx 10$ ($0 < y < 0.5$), where y is the fractional saturation of haemoglobin S with carbon monoxide^{7,8}. Assuming that the perturbation responsible for the change in oxygen affinity alters neither the degree of cooperativity nor the delay time for the completely deoxygenated molecule, we can use the result for carbon monoxide to calculate the minimum decrease in p_{50} required to produce a given increase in delay time. Table 1 summarises the results of the above calculations. As solubility is a frequently used assay for gelation, and is related to the delay time by $d \log t_d / d \log c_s = 30-40$ (refs 7, 26, 30), the increase in solubility corresponding to the delay time increase is also shown.

Table 1 suggests that the values of x , Δc and Δp_{50} required to produce a 'cure' of S/S disease are large and will be difficult to achieve. To obtain 75% saturation of a perfect inhibitory site, and simultaneously maintain a low free inhibitor concentration to minimise toxicity, a molecule with a high binding constant is required. A decrease in intracellular haemoglobin concentration of $75-110 \text{ g l}^{-1}$ might be accomplished with a reducti-

Table 1 Clinical course, gelation delay time and requirements for methods of inhibition

Condition	Clinical course relative to S/S disease	Red cell composition			t_d	$\frac{c_s}{c_s^0}$	x	Δc (gl^{-1})	Δp_{50} (mm Hg)
		X_S	X_A	X_F	$t_d(\text{S/S})$	$\frac{c_s}{c_s^0}$			
S/ β^+ -thal.	Less severe	0.85–0.7	0.15–0.3	0	10^{-10^2}	1.06–1.17	0.20–0.37	15–40	>4–7
S/HPFH	Much less severe	0.8–0.7	0	0.2–0.3	10^3-10^4	1.19–1.26	0.50–0.60	40–80	>10–13
A/S trait	No disease	0.4	0.6	0	10^6	1.4–1.6	0.75	75–110	>17

X_i values ($i = \text{Hb S, Hb A, Hb F}$) are mol fractions; $t_d/t_d(\text{S/S})$ is the ratio of the mixture delay time for each composition to the delay time for pure deoxyhaemoglobin S at the same total haemoglobin concentration and is obtained from the data in Fig. 1; c_s/c_s^0 is the ratio of deoxyhaemoglobin S solubility in the presence of inhibitor to deoxyhaemoglobin S solubility without inhibitor; x is the fractional saturation of the inhibitory site required to produce the delay time increase; Δc is the required decrease in intracellular haemoglobin concentration, assuming a mean intracellular concentration of 340 g l^{-1} in S/S disease; Δp_{50} is the required decrease in partial oxygen pressure at 50% saturation with oxygen. For a given increase in the delay time the minimum Δp_{50} can be calculated from the experimental result on solutions partially saturated with carbon monoxide that $d \log t_d/dy \approx 10^{7.8}$, and from the Hill equation, $\log(y/1-y) = n \log(p/p_{50})$. It is assumed that $n = 2.6$ and $p_{50} = 26 \text{ mm Hg}$ for haemoglobin S during the delay period, where a negligible amount of polymerisation has occurred.

in haemoglobin synthesis, by, for example, introducing an iron deficiency (the ideal result would be a ‘switch’ from sickle cell disease to a much less severe anaemia)^{11,31}. An equivalent increase in cell volume (22–32%) would be preferable, as it would presumably result in less interference with oxygen delivery. Increasing oxygen affinity is the most difficult strategy to evaluate. A realistic calculation would require detailed considerations of the kinetics of intracapillary deoxygenation. There is, moreover, insufficient data to evaluate the influence of homeostatic responses that maintain oxygen delivery. A decrease in tissue p_{O_2} to increase oxygen extraction, would, for example, partially buffer the inhibitory effect of an increase in oxygen affinity³². Note, however, that complete suppression of 2,3-diphosphoglycerate synthesis could lower the p_{50} by as much as 10 mm Hg^{33,34}. Finally, we emphasise that the calculations in Table 1 consider only changes in single variables. Both molecular and physiological responses to a particular perturbation could result in simultaneous changes in all three. However, the comparisons in Table 1 suggest that therapeutically significant kinetic inhibition of intracellular gelation could be accomplished by any one of the three approaches.

Many gelation inhibitors have been proposed as specific drugs for sickle cell disease³⁵. The results in Table 1 should provide a useful framework for assessing the therapeutic potential of these inhibitors, particularly in analysing the problem of gelation inhibition versus toxicity. Because very large doses of any single drug would be necessary to obtain a therapeutic effect in patients, it will probably be necessary to employ several drugs simultaneously, taking advantage of the multiple methods by which the delay time of intracellular gelation can be increased.

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Initiation activity of EMC virus RNA, binding to initiation factor eIF-4B and shut-off of host cell protein synthesis

PICORNAVIRUSES infecting susceptible animal cells inhibit synthesis of host proteins and direct the cells to synthesise viral proteins¹. The mechanism for the shut-off of host cell protein synthesis is not understood, although several experiments indicate that initiation of host protein synthesis is specifically inhibited¹. When initiation of protein synthesis is reduced by exposing cells infected by picornaviruses to hypertonic medium, synthesis of viral proteins is greatly favoured over that of host proteins^{2,3}. This suggests that viral templates are more successful in initiation than host templates³. Competition between picornavirus RNA and host mRNAs has been shown directly by *in vitro* translation experiments^{4,5}. When these RNAs are added together in saturating amounts to a cell-free protein-synthesising system, viral RNA is preferentially translated over host mRNA^{4,5}. This competition can be relieved by addition of initiation factor eIF-4B (previously designated IF-M₃)⁶. This has suggested that eIF-4B may be present in limiting amounts in the translation assays, and that viral and host templates compete for available factor⁶. This observation is somewhat surprising, as the 5'-terminal cap sequence m⁷G^{5'}pppXmp... is involved in the binding of mRNA to eIF-4B, and picornavirus RNA is not capped (see refs 7 and 8). It has been found that other nucleotide sequences outside the cap participate in this reaction⁹, but the presence of 5'-terminal m⁷G in mRNA promotes interaction

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with eIF-4B and the initiation process^{7,8}. Therefore, templates with a 'capped' 5'-terminal nucleotide bind to ribosomes more rapidly than their unmethylated counterparts^{10,11}. We have shown previously that eIF-4B interacts with the RNA of encephalomyocarditis (EMC) virus, a picornavirus, in a nitrocellulose filter binding assay⁷. It has also been shown that eIF-4B is required for *in vitro* translation of EMC virus RNA¹². In the present report we have examined the basis for the competition between EMC virus RNA and a cellular mRNA at the molecular level. EMC virus RNA binds to ribosomes at a rate similar to that of capped mRNAs, suggesting that features other than the 5'-terminal m⁷G are responsible for the binding of this viral RNA. By measuring the relative affinity of globin mRNA and EMC virus RNA for eIF-4B, we have found that the viral RNA has a greater affinity for this initiation factor than globin mRNA. This high affinity for eIF-4B could explain the fast rate of initiation of the viral RNA and the shut-off of host cell protein synthesis after picornavirus infection.

The rate of binding of EMC virus RNA to ribosomes was examined in cell-free systems prepared from HeLa cells and reticulocyte lysate¹³. Labelled EMC virus RNA obtained from virions or from infected cells formed initiation complexes by binding to ribosomal subunits (Fig. 1). As was previously described^{7,10}, the cell extracts were incubated for 2 min with the inhibitor of elongation sparsomycin before addition of viral RNA. In the presence of sparsomycin, ribosomes were stuck to the initiation site of cellular mRNA. Therefore, the cellular mRNA was effectively blocked from reinitiating. This procedure avoided competition between the trace amount of the highly radioactive viral RNA added and the larger amounts of cellular mRNA present in the extracts, and enabled us to measure the kinetics of binding of viral RNA to ribosomes without interference from competitor mRNA species (Fig. 2).

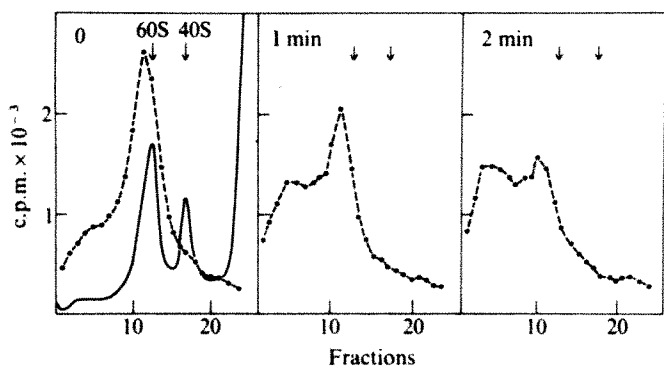


Fig. 1 Binding of EMC RNA to HeLa cell ribosomes (●, c.p.m. $\times 10^{-3}$). Labelled EMC virus was obtained from infected HeLa cells incubated with ³H-uridine, and the viral RNA purified as described previously⁷. Cytoplasmic extracts were prepared from HeLa cells as described previously¹³. Each incubation contained in a final volume of 0.1 ml: 70 μ l cell extract, 5 μ l 2 mM sparsomycin, 1 μ g reticulocyte ribosomal RNA, 20 μ l of the energy mix for protein synthesis described previously¹², amino acids at 0.05 mM, K OAc at 0.12 M and Mg(OAc)₂ at 2.5 mM final concentrations. After 2 min incubation at 30 °C, 5 μ l EMC ³H-RNA (20,000 c.p.m.) were added for the time indicated. The incubations were terminated by adding 4 volumes ice-cold 0.5 M NaCl, 30 mM Mg OAc₂ and 20 mM HEPES-KOH, pH 7.4, and applied to 15–30% sucrose gradients in the same buffer. After 17 h centrifugation at 23,000 r.p.m., the gradients were fractionated through a recording spectrophotometer. An identical A₂₆₀ profile (solid line) was obtained for all gradients and is reported in the first panel only. The gradient fractions were counted after addition of 9 ml Scintiverse (Fisher). In the left panel, the incubation has been terminated immediately after addition of the RNA and the EMC RNA is not bound to ribosomes. In the other panels, the binding of EMC RNA to ribosomes is shown by the appearance of a peak sedimenting faster than the unbound EMC RNA. To determine the amount of bound EMC RNA as a per cent of input RNA (Fig. 2), the areas of the peaks corresponding to free and bound EMC RNA were measured with a Du Pont 310 Curve Resolver.

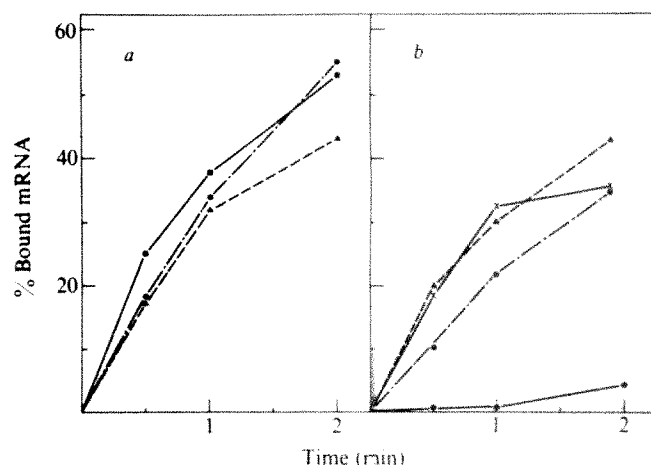


Fig. 2 Rate of binding to ribosomes of EMC RNA and of other viral mRNAs, in HeLa cell extract (a) and in a reticulocyte lysate (b). EMC ³H RNA was obtained from virions (●) as described previously⁷, or from infected HeLa cells (■) according to Fernandez-Munoz and Lavi²⁸, as follows: the cells were infected at room temperature as outlined previously⁷; after 30 min, actinomycin D was added to a final concentration of 5 μ g ml⁻¹. After 90 min, 5 μ Ci ml⁻¹ ³H uridine were added and the incubation continued for 2 h. The cells were collected, washed and homogenised as described previously¹³. The cell extract was made 0.5% in NP-40 and 30 μ g ml⁻¹ in dextran sulphate, centrifuged for 5 min to remove the nuclei, and adjusted to a final concentration of 20 mM EDTA and 1% sodium dodecylsulphate. The 35S EMC RNA was purified by three successive centrifugations on 15–30% sucrose gradients as described previously⁷. The EMC RNA had a specific activity of about 200,000 c.p.m. per μ g RNA. Other viral mRNAs were prepared as described previously for vesicular stomatitis virus polysomal mRNA⁷ (▲), and for methylated (×) and unmethylated (♦) vaccinia mRNA¹¹. The rate of binding of the different RNAs was measured as described in Fig. 1. The input of VSV mRNA was 10,000 c.p.m. per incubation. The binding assays with vaccinia mRNA have previously been described.¹¹

To resolve EMC virus RNA bound to ribosomes from unbound viral RNA, reaction mixtures were centrifuged through sucrose gradients containing 0.5 M NaCl (Fig. 1). In these conditions, single ribosomes were dissociated, whereas initiation complexes were stable¹⁰. Moreover, nonspecific association of cytoplasmic proteins with EMC virus RNA was reduced, allowing a better separation of the RNA bound to ribosomes from unbound RNA¹⁴.

In a reaction kept at 0 °C most of the EMC virus RNA not bound to ribosomes sedimented at about 70S (Fig. 1). After 1 min incubation, a peak sedimenting at about 105S was seen, and after 2 min incubation this peak was approximately equal in height to the peak of unbound EMC virus mRNA. As these peaks were incompletely resolved, it was necessary to use a Du Pont curve resolver to calculate the percentage of input RNA bound to ribosomes (see Fig. 1). This difficulty in determining the percentage of RNA bound to ribosomes was only encountered with the large picornavirus RNA. Binding of one ribosome to this RNA did not increase its sedimentation sufficiently to allow complete separation between bound and unbound RNA, as is the case with smaller mRNAs^{7,11}.

The rate of binding of EMC virus RNA to ribosomes was similar to that of capped viral mRNAs obtained from cells infected with vesicular stomatitis virus (VSV) or transcribed *in vitro* from vaccinia virions^{7,11}. The rate of binding was linear for 1 min and decreased thereafter; very little additional binding was observed after 2 min of incubation (not shown). Unmethylated vaccinia mRNA bound to reticulocyte ribosomes very slowly (Fig. 2b), as previously reported¹¹. This result was also obtained in the HeLa cell-free system (not shown). The rate of binding of cellular histone mRNA was determined in separate experiments (L. A. Weber and C. B., unpublished observation) and was found to be similar to that of the viral mRNAs. Histone mRNA is also capped¹⁵.

These results indicate that the uncapped EMC virus RNA interacts with initiation factors and ribosomal subunits to form initiation complexes at a rate comparable to that of capped cellular and viral mRNAs. As the interaction of mRNAs with initiation factor eIF-4B may play an important part in determining the rate of initiation, we measured the binding of viral RNA and globin mRNA to purified eIF-4B. The relative affinity of an RNA for this factor was determined by competition-binding experiments (Table 1)⁷. Competition-binding is the most sensitive and accurate method for comparing the relative affinity of two mRNAs for a given initiation factor, and in the conditions used only high affinity interactions were measured. This was obtained by reacting eIF-4B with labelled mRNA at low concentrations, such that only 25–30% of the input mRNA was bound (Table 1). Different unlabelled mRNAs were then added to determine their relative effect as competitors in the binding with eIF-4B. The validity of this assay was shown by experiments with globin mRNA (Table 1). The same preparation of globin mRNA purified by oligo(dT)-cellulose chromatography and gradient centrifugation⁷ was either labelled with ¹²⁵I or used unlabelled as a competitor. A 60% binding competition was observed after addition of a 2.9-fold excess of unlabelled mRNA. The increased concentration of mRNA in this reaction favoured its association with eIF-4B compared with the reaction containing unlabelled mRNA only, and consequently the apparent competition was less than the theoretical level expected. However, the important point shown by the experiments of Table 1 is that on a molar basis EMC virus RNA competed with labelled VSV and globin mRNA about 10-fold and 100-fold more effectively than unlabelled globin mRNA. Even on a microgram basis, EMC virus RNA was more effective than globin mRNA in competing with labelled globin mRNA, as 0.06 µg EMC-RNA competed almost as effectively as 0.42 µg unlabelled globin mRNA.

These findings provide direct evidence that eIF-4B was not bound randomly to RNA, in proportion to the total mass of nucleotides, but that the binding was occurring at specific site(s). Additional evidence for this specific interaction was obtained by using ribosomal RNA instead of mRNA as a competitor in the binding reactions and by directly determining the affinity of EMC virus RNA and globin mRNA for eIF-4B. Ribosomal

Table 1 EMC virus RNA competition for binding of VSV mRNA and globin mRNA to purified reticulocyte initiation factor eIF-4B (IF-M₃)

Competitor RNA	µg	pmol	mRNA bound (c.p.m.)		% Competition
			³ H-VSV mRNA	¹²⁵ I globin mRNA	
None	—	—	3713	—	—
Globin mRNA	0.25	1.1	2593	—	30
EMC RNA	0.94	0.3	1612	—	57
None	—	—	—	4702	—
Globin mRNA	0.42	1.8	—	1874	60
EMC RNA	0.06	0.02	—	2241	52

Incubations were carried out at 23 °C for 5 min in a 50 µl reaction mixture and contained 20 mM Tris-HCl, pH 7.4, 0.1 M KCl, 1 mM dithiothreitol, 1.6 µg eIF-4B plus 14,620 c.p.m. ³H-VSV mRNA (3.6 pmol) or 0.2 µg eIF-4B plus 14,700 c.p.m. of ¹²⁵I-globin mRNA (62 pmol), and amounts of competing unlabelled globin mRNA or EMC RNA as indicated. Reactions were stopped by dilution to 3 ml cold buffer (20 mM Tris-HCl, pH 7.4, and 100 mM KCl), and protein complex formation measured by collection of the radioactivity on a nitrocellulose filter as previously reported⁷. Blanks for ³H-VSV or 736 c.p.m. for ¹²⁵I-globin mRNA, representing background radioactivity, were subtracted from each value. Globin mRNA was iodinated according to a modification²⁶ of the procedure of Melnick²⁷. A molecular weight of 2.3 × 10⁵ has been estimated for EMC RNA³⁰.

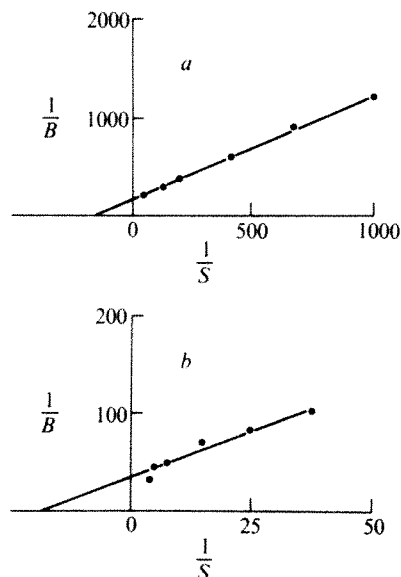


Fig. 3 Binding affinity of EMC RNA (a) and globin mRNA (b) for initiation factor eIF-4B. The binding assays were carried out as described in Table 1, with 0.05 µg eIF-4B per assay and either EMC ¹²⁵I RNA (specific activity 4 × 10⁶ c.p.m. µg⁻¹) or globin ¹²⁵I mRNA (specific activity 1.8 × 10⁶ c.p.m. µg⁻¹). The reciprocals of the bound RNA (1/B) compared with the input RNA (1/S) are shown. Concentrations are in pmol. The dissociation constant (K_D) is given by the intercept of the lines with the abscissa²⁹. The K_D for EMC RNA is 1.43 × 10⁻¹⁰ mol l⁻¹ and that for globin mRNA is 1.08 × 10⁻⁹ mol l⁻¹.

RNA was found to be a poor competitor of the binding of mRNA to eIF-4B as only 100-fold greater amounts of rRNA competed appreciably in the reaction (data not shown). The affinity of EMC virus mRNA and globin mRNA for eIF-4B was determined by reacting a constant amount of initiation factor with decreasing amounts of RNA. The results are shown as a double-reciprocal plot in Fig. 3. The experimental points, and in particular those presumably measuring high affinity interactions at high RNA dilutions, fall on a line. This enabled us to measure the dissociation constants of complex formation by a graphic method (see legend to Fig. 3) and to establish that the affinity of EMC virus RNA for eIF-4B is approximately one order of magnitude higher than that of globin mRNA.

The present study is the first report of a determination of binding constants (K_D) for mRNA/eIF-4B interaction. Note that these K_D values are averages and that no assumption has been made in their calculation regarding specific sequences involved, number of binding sites, or configurational properties of the RNA or protein.

However, the significance of the binding of mRNA to eIF-4B cannot be functionally evaluated as with the presently available assays it is not possible to demonstrate that these complexes are biologically active.

The high affinity of EMC virus RNA for eIF-4B is a property of the viral RNA, which most likely determines its rate of initiation *in vitro* and its ability to compete more successfully than capped mRNAs *in vivo*. An increased rate of initiation has been shown after enzymatic addition of an m⁷G⁵p cap to the uncapped RNA of satellite tobacco necrosis virus RNA¹⁶. Addition of a cap also increases the affinity of this viral RNA for eIF-4B⁹. Picornavirus RNA competes more successfully than not only host mRNA but also other capped viral RNAs, such as VSV mRNA. In cells infected with VSV and superinfected with poliovirus, host and VSV mRNAs are translated poorly compared with poliovirus RNA^{17,18}. In these conditions, synthesis of VSV mRNA continues, but this RNA does not associate with polysomes¹⁸. Although other interpretations are possible, these observations could be explained by the high affinity of picornavirus RNA for eIF-4B reported here. The

structural basis for this high affinity is not known. Picornavirus RNA has a rather unique structure with a protein covalently bound to the 5'-terminal nucleotide^{19,20}. However, it seems unlikely that this protein plays a part in the interaction with eIF-4B and in the competition between picornavirus and host mRNA, as the viral mRNA synthesised in infected cells does not have a protein bound to the 5'-terminal nucleotide²¹⁻²³. Nucleotide sequences shared by virion RNA and by viral mRNA are presumably involved in the interaction with eIF-4B.

Initiation factors are found bound to 40S ribosomal subunits²⁴, are released after formation of initiation complexes and recycle in order to function catalytically in initiation¹³. The high affinity of picornavirus RNA for eIF-4B may interfere with the recycling of this factor. The viral RNA may form a more stable complex with eIF-4B and this may prevent the factor from functioning with host mRNAs. Golini *et al.*⁶ have proposed a similar explanation for their observation that addition of eIF-4B relieves the competition between EMC virus RNA and host mRNA for *in vitro* translation. The present study is consistent with this interpretation in that EMC virus RNA has a significantly higher binding affinity for eIF-4B than globin mRNA, a natural substrate for this initiation factor. However, binding of other initiation factors to EMC virus RNA has been reported⁷, and interaction with these other factors also plays an important part in the translation of this viral RNA²⁵. Therefore, it remains to be established whether the high affinity of picornavirus RNA for eIF-4B by itself accounts for the shut-off of host protein synthesis in virus-infected cells.

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Does ultraviolet light enhance postreplication repair in mammalian cells?

D'AMBROSIO AND SETLOW reported an apparent enhancement of postreplication repair in Chinese hamster V79 cells¹. A first exposure of cells to ultraviolet (UV) light was said to make it possible, after re-exposure to UV, for the cells to join the DNA together to form high molecular weight DNA more rapidly than if the cells had not been previously exposed. They irradiated cells with 2.5 J m⁻² of UV 2 h before a second exposure to 5.0 J m⁻², and then pulse-labelled the cells with ³H-thymidine before chasing for 1 h. When analysed by alkaline sucrose gradient velocity sedimentation, the DNA of these cells showed a profile of radioactivity very different from that of cells irradiated only once with 5.0 J m⁻² before pulse-labelling and chasing. For cells irradiated twice the peak of labelled DNA was at a position corresponding to a higher molecular weight than the peak for cells irradiated once. I report here results confirming the data of D'Ambrosio and Setlow, but use of an alternative method of data presentation suggests that interpretations other than the one assumed by D'Ambrosio and Setlow are equally possible or maybe more likely.

When Chinese hamster cells were irradiated with 2.5 J m⁻² and, 2 h later, with 5.0 J m⁻² of UV light and then pulse-chased, their ³H-labelled DNA showed two peaks of radioactivity on alkaline sucrose gradient profiles plotted as percentage of total counts in each gradient (Fig. 1). One peak (fraction 18) was near the peak exhibited by the DNA of unirradiated cells receiving the same protocol, and the other was at a position of lower molecular weight (fraction 13). Cells treated with 7.5 J m⁻² and then pulse-chased showed a major ³H-labelled DNA peak corresponding to a low molecular weight (fraction 8) and a broad shoulder extending into the position of the peak for cells irradiated with 2.5+5.0 J m⁻². The ³H-labelled DNA from cells treated with 5.0 J m⁻² peaked at the same fraction as the minor peak of cells irradiated with 2.5+5.0 J m⁻² (fraction 13); a prominent shoulder at high molecular weight (fractions 15-18) was also present. The ³H-labelled DNA from cells treated only with 2.5 J m⁻² exhibited an alkaline gradient profile which was intermediate between that of DNA from controls and DNA from cells treated with 2.5+5.0 J m⁻² (Fig. 1). These results confirm the data of D'Ambrosio and Setlow.

D'Ambrosio and Setlow based their interpretation of the data on profiles plotted as percentage of total radioactivity in each gradient. When the same data are presented in a different form another interpretation is possible. Profiles plotted as total radioactivity per fixed number of cells (Fig. 2) demonstrate the inhibition of DNA synthesis caused by each treatment; this information is lacking in graphs plotted as percentage of total radioactivity in each gradient (Fig. 1). Figure 2 shows that the total radioactivity in fractions 18-21 from ³H-labelled DNA of cells irradiated with 2.5+5.0 J m⁻² was only slightly higher than the radioactivity in the same fractions from the DNA of cells treated only with 7.5 J m⁻², and lower than that in cells treated only with 5.0 J m⁻². The profile from cells that received only 2.5 J m⁻² shows the greatest amount of radioactivity in these fractions (except for control) but is still severely depressed from the control. When, as in Fig. 2, the data are plotted to show the large inhibitions of DNA synthesis that occur after UV irradiation, the significance of peaks that appear distinctive in Fig. 1 becomes questionable.

The data presentation in Fig. 2 also poses the question, whether the status of DNA replication in cells 2 h after 2.5 J m⁻² of UV is the same as in unirradiated cells? To answer this question, a similar experimental protocol was used but without a chase after the ³H-thymidine pulse. When the ³H-labelled DNA from these pulse-labelled cells was analysed on alkaline sucrose gradients (Fig. 3) their principal peaks were, as expected, at lower molecular weights than unirradiated cells. The ³H-

labelled DNA from cells exposed to $2.5 + 5.0 \text{ J m}^{-2}$ had a minor but significant peak corresponding to a molecular weight even higher than that of the main peak of control ^3H -labelled DNA. A very prominent minor peak at about the same point was also evident in the profile of ^3H -labelled DNA from cells exposed only to 2.5 J m^{-2} at 2.5 h before the pulse with ^3H -thymidine. The appearance of these peaks at high molecular weight is reproducible.

Analysis of profiles of both pulse-labelled and pulse-chased DNA leads to the following conclusions:

(1) Cells exposed to 2.5 J m^{-2} have not recovered from the damage in their DNA by 2.5 h after irradiation; the overall rate

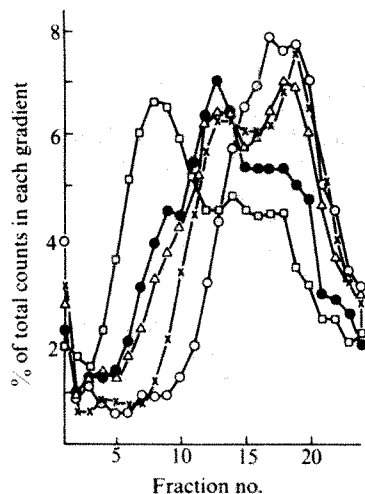


Fig. 1 Alkaline sucrose gradient profiles of ^3H -labelled nascent DNA from Chinese hamster cells. Chinese hamster V79 cells, maintained in this laboratory for several years, were grown in Falcon plastic Petri dishes in Eagle's minimum essential medium (MEM) containing 15% fetal calf serum (Gibco). Exponentially growing cells incubated overnight in $0.01 \mu\text{Ci ml}^{-1}$ of ^{14}C -thymidine (57 Ci mol^{-1} ; Schwarz/Mann) were washed twice with Puck's phosphate-buffered saline G^2 and exposed to various fluences of UV light from a mercury germicidal lamp with an incident intensity of $1.3 \text{ J m}^{-2} \text{ s}^{-1}$. After irradiation, MEM was placed back on the cultures for 30 min, after which it was replaced with MEM containing $20 \mu\text{Ci ml}^{-1}$ of ^3H -thymidine ($4 \times 10^{-7} \text{ M}$). After 10 min of incubation, the radioactive medium was removed. Fresh MEM containing 10^{-4} M thymidine and 10^{-5} M deoxycytidine was added to the cells, which were incubated (chased) for 1 h before the reaction was stopped with ice-cold 0.15 M sodium chloride, 0.015 M sodium citrate (SSC). The cells were washed once with SSC, scraped into it, and a sample containing about 10^5 cells (in less than 0.5 ml) was irradiated with $1,000 \text{ rad}$ of X rays to enhance lysis³ and then added directly to 0.5 ml of lysis solution (0.5 M NaOH, 0.02 M EDTA, 0.1% NP40 [non-ionic detergent, Shell]) on top of 30 ml of preformed 5–20% alkaline sucrose gradients (0.1 M NaOH, 0.9 M NaCl, 0.01 M EDTA). Lysis proceeded in the dark at 25°C for 30 min and the gradients were then centrifuged in an SW27 rotor (Beckman) at $27,000 \text{ r.p.m.}$ for 3 h or at $12,000 \text{ r.p.m.}$ for 15 h. This gradient system has been calibrated with phage λ DNA and phage T_4 DNA as described⁴. About 25 equal-weight fractions were collected, $100 \mu\text{g}$ of calf thymus DNA were added to each fraction, and all of the DNA was precipitated with ice-cold 6 M HCl plus 6% sodium pyrophosphate. Each fraction was then filtered through GF/C filters (Whatman) presoaked with 4% HClO_4 . The filters were washed sequentially with ice-cold 4% HClO_4 , 70% alcohol, and 100% alcohol and dried; the ^{14}C and ^3H on each filter were determined with a Packard liquid scintillation spectrometer. $\circ$, Unirradiated cells; $\times$, cells irradiated only with 2.5 J m^{-2} at 2.5 h before pulse-labelling; Δ , cells irradiated with 2.5 J m^{-2} at 2.5 h before pulse-labelling and with 5.0 J m^{-2} 0.5 h before pulse-labelling; $\bullet$, cells irradiated only with 5.0 J m^{-2} at 0.5 h before pulse-labelling; $\square$, cells irradiated only with 7.5 J m^{-2} at 0.5 h before pulse-labelling. Sedimentation is from left to right. Note that the ordinate is percentage of total counts in each gradient. The majority of the ^{14}C -labelled parental DNA sedimented to the bottom of the gradient in all cases.

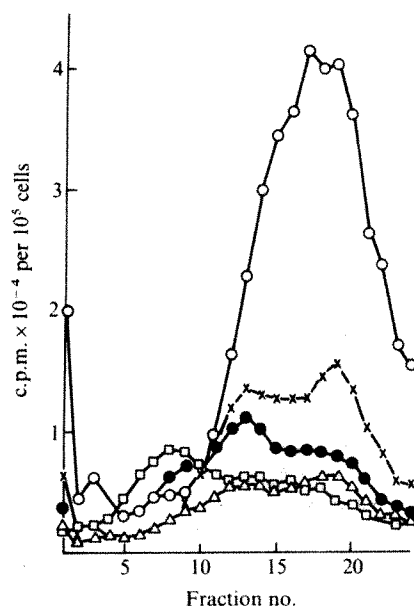
of DNA synthesis is still depressed by 25–50%. More importantly, the status of nascent DNA molecules in these cells is not the same as in controls; although the molecular weight of most nascent DNA molecules in treated cells is lower than in control cells, a significant number of them have a higher molecular weight (fractions 12–14, Fig. 3).

(2) When cells are exposed to 2.5 J m^{-2} of UV and then 2 h later to 5.0 J m^{-2} , 30 min after the second exposure (at the time of pulse-labelling), their nascent DNA molecules more closely resemble the nascent DNA in cells treated only with 5.0 J m^{-2} than in cells treated only with 7.5 J m^{-2} (Fig. 3). The gradient profile of DNA from cells treated with $2.5 + 5.0 \text{ J m}^{-2}$ also resembles that of DNA from cells treated only with 2.5 J m^{-2} , in that a significant amount of nascent DNA has a much higher than average molecular weight after both treatments (peak at fraction 14, Fig. 3). These results imply that 2 h after cells have been exposed to 2.5 J m^{-2} UV, many elongating DNA chains are still temporarily blocked but others have circumvented the lesions and extended to form molecules that are larger than average but still not of parental size. When the cells are subsequently exposed to 5.0 J m^{-2} , these intermediate-sized nascent DNA molecules remain as a source for further chain elongation.

(3) When pulse-chase experiments are performed, the peaks at high molecular weight (fraction 18, Figs 1 and 2) in gradient profiles of DNA from cells treated with 2.5 and 5.0 J m^{-2} probably represent (i) DNA molecules formed by the elongation of the intermediate molecular weight molecules (peak at fraction 14, Fig. 3) observed in pulse-labelled cells and (ii) DNA molecules formed by elongation of low molecular weight molecules (fraction 5, Fig. 3), as in cells exposed to 5.0 J m^{-2} alone. Elongation of intermediate molecular weight molecules accounts for the greater amounts of radioactivity at high molecular weight (fractions 18–21, Figs 1 and 2) in the DNA of cells treated with $2.5 + 5.0 \text{ J m}^{-2}$ over the radioactivity in the same fractions in cells exposed only to 7.5 J m^{-2} .

Thus, the effects of 5.0 J m^{-2} of UV light on DNA synthesis and on maturation of nascent Chinese hamster V79 DNA are different in cells that were irradiated 2 h earlier with 2.5 J m^{-2} than in previously unirradiated cells, but these differences can be explained entirely by the damaging effects of the first exposure, which cause an abnormal distribution of sizes of nascent DNA molecules at the time of irradiation with 5.0 J m^{-2} . These considerations make it unnecessary to invoke the existence of an

Fig. 2 Same data as Fig. 1, except that the ordinate measures total counts per 10^5 cells.



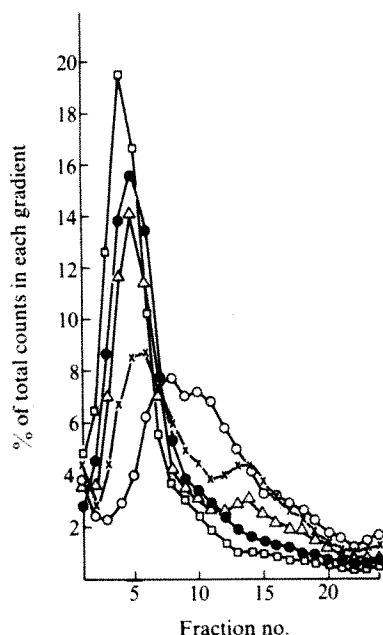


Fig. 3 Alkaline sucrose gradient profiles of ^3H -labelled nascent DNA from Chinese hamster cells. Protocol and symbols the same as Fig. 1, except that the cells were pulse-labelled but not chased before analysis. The reaction was stopped by addition of ice-cold SSC.

enhanced postreplication repair system to explain the altered pulse-chase profiles observed for the DNA of mammalian cells exposed to two irradiations of UV.

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Identification of the haem ligands of cytochrome b_{562} by X-ray and NMR methods

X-RAY crystallography and nuclear magnetic resonance (NMR) are the two most powerful physical techniques for studying macromolecular structure. They are often used to complement one another in the study of a particular molecule. Crystallographic results have been of tremendous help in the NMR peak assignments which are subsequently used to obtain structural information in solution^{1,2}. We report the combined use of both X-ray crystallography and NMR to identify MET-7 and HIS-106 as the two ligands of the haem iron of cytochrome b_{562} .

Cytochrome b_{562} is a cytoplasmic component of *Escherichia coli*. It contains 110 amino acids of known sequence³ and a non-covalently bound haem group⁴. The sequence is shown in Fig. 1. The molecule contains two histidines at positions 7, 35 and 61. The identity of the haem ligands was previously unknown, although spectral similarities to the b -type cytochromes and sequence homology to myoglobin suggested that they were histidine⁵.



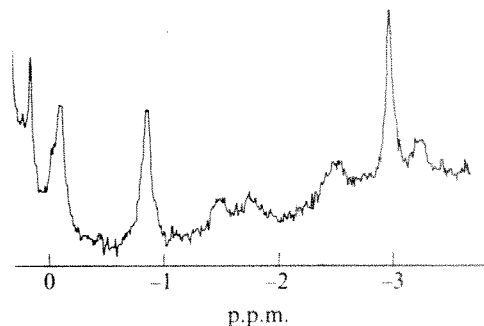
Fig. 1 Amino acid sequence of cytochrome b_{562} (ref. 3). Haem ligands, MET-7 and HIS-106 are circled. The other histidine and two remaining methionine side chains are underlined.

We have been studying the structure of cytochrome b_{562} using X-ray methods. The triclinic crystals contain two molecules in a unit cell of dimensions: $a = 33.71 \text{ \AA}$; $b = 50.48 \text{ \AA}$; $c = 32.73 \text{ \AA}$; $\alpha = 102.76^\circ$; $\beta = 86.55^\circ$; $\gamma = 106.70^\circ$ (ref. 6). The two molecules are related by a non-crystallographic twofold axis nearly perpendicular to the b axis⁷. We have obtained a single uranyl acetate derivative containing one major binding site and four minor sites per unit cell. An electron density map has been calculated at $2.5 \text{ \AA}$ resolution based on X-ray measurements including anomalous scattering from native and derivative crystals. Details of the data collection procedure, derivative preparation, heavy atom refinement and map calculation will be published elsewhere.

The map was averaged about the non-crystallographic twofold axis and plotted on a mini-map at a scale of $1.0 \text{ cm \AA}^{-1}$. The haem group was clearly seen and portions of the polypeptide chain could be traced. However, the density merged at several points, making it difficult to trace the chain unambiguously. The map was then transferred to a Richards box at a scale of $2 \text{ cm \AA}^{-1}$ in order to build a Kendrew model into the density.

It was relatively easy to identify the ligand on one face of the haem group as HIS-106 and to fit its side chain and those of its

Fig. 2 High field region of the 270 MHz ^1H -NMR spectrum of ferrocyanide b_{562} (0.2 ml 1.5 mM in D_2O , nominal pH = 7.0, 40°C). The spectrum was obtained in a Bruker 270 MHz spectrometer. The solvent resonance was suppressed^{19,20} and 7,000 transients were accumulated in a Nicolett 1180 computer. The sample was reduced with minimal amounts of crystalline sodium dithionite under argon. Dioxan was used as internal standard but all the chemical shifts are quoted in parts per million (p.p.m.) from 2,2-dimethyl-2-silapentane-5-sulphonate.



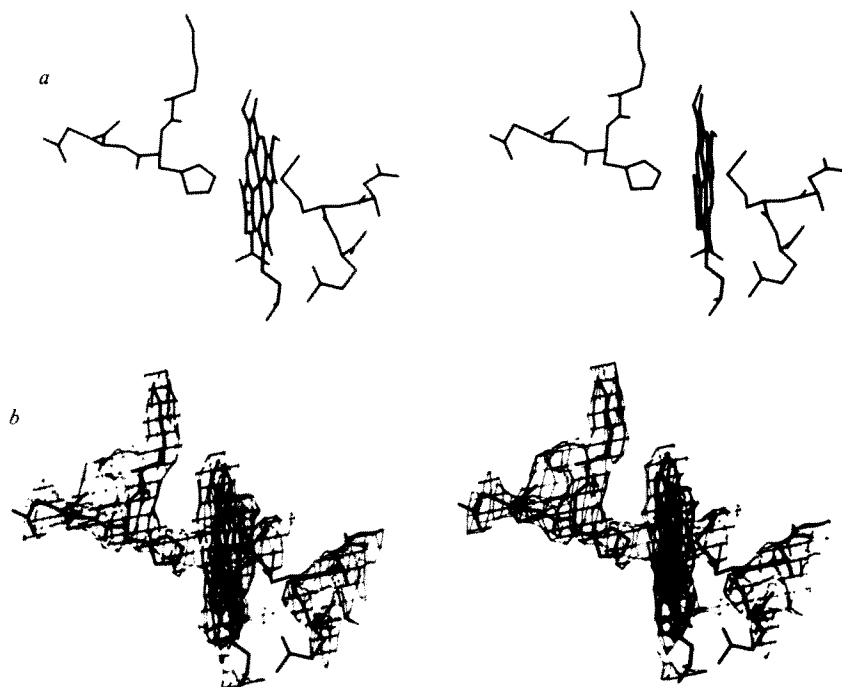


Fig. 3 Stereo photographs showing the haem group and the two ligands with adjacent side chains. The sequence asp⁶-met⁷-gln⁸ is on the right side with gln⁸ at the bottom. The sequence lys¹⁰⁵-his¹⁰⁶-gln¹⁰⁷ is on the left with lys¹⁰⁵ at the top. *a*, Skeletal model only; *b*, skeletal model superimposed on the electron density. The MMS-X molecular graphics computer developed at Washington University was used to produce the photograph. This system was not yet available during the model building stages described in the text.

immediate neighbours into the density. On the other face of the haem group, however, it was not possible to identify the side-chain ligand, and either histidine or methionine (or indeed some other side chain) could be placed in the density.

We decided that an independent method was needed to confirm the fifth ligand and to identify the sixth ligand before the model building could proceed. NMR is a non-destructive method particularly suited for this type of study. A considerable number of cytochromes in which the iron is ligated with either one histidine and one methionine⁸ or two histidines⁹⁻¹² have been characterised using NMR. The chemical shifts of the proton NMR spectrum of a methionine residue ligated to the low spin ferrous ion of a *c* type haem give a very characteristic pattern^{13,14}. These chemical shifts have been used to identify methionine as the sixth ligand of several cytochromes^{15,16}. The electron proton resonance (EPR) spectra¹⁷ of cytochromes *c*₃ from *Desulphovibrio* sp. and the absence of such chemical shifts in the NMR spectra of the low spin ferrous form of these cytochromes¹⁰⁻¹² prove that both the fifth and the sixth iron ligands are histidines. Similarly, the NMR spectra of low spin ferrocyanochrome *b*₅, which has two histidine ligands¹⁸, do not show the typical features of a methionine residue bound to the haem iron⁹.

Figure 2 shows the high field region of the high resolution proton NMR spectrum of reduced cytochrome *b*₅₆₂. The three protons intensity singlet at -2.98 p.p.m. is unequivocally assigned to the methyl group of a methionine coordinated to the haem iron^{13,14}. The one proton intensity resonances at -1.46 p.p.m., -1.75 p.p.m., -2.48 p.p.m. and -3.22 p.p.m. are assigned to the β - and γ -methylene protons of this residue. The one proton intensity singlet at 0.11 p.p.m. is in the region expected for one of the protons of histidine residues bound to low spin haem iron^{15,16} and supports earlier evidence⁵ for the nature of the fifth ligand (HIS-106).

Once it was known that the second haem ligand was methionine, it was relatively easy to identify which of the three it was. Short sequences of polypeptide chain corresponding to each methionine and two residues on either side were built. Each was tested in the density, and one sequence containing MET-7 fit the density much better than the others. The chain was then built into the density from MET-7 toward the N-terminus and forward to about residue 25. The conformation of the chain in this region consists of about four turns of α -helix. Figure 3 shows the model of the haems MET-7 and HIS-106 and their adjacent residues superimposed on the electron density map.

The model of cytochrome *b*₅₆₂ has been completed, but there are still regions of possible ambiguity in the interpretation. The electron density map is presently being refined by the method of molecular replacement²¹. The results and complete structure will be reported elsewhere.

Cytochrome *b*₅₆₂ is the first *b*-type cytochrome shown to contain both histidine and methionine as haem ligands. In contrast to cytochrome *c* the methionine occurs before histidine in sequence. However, cytochrome *c*₃ has *c*-type haems ligated by two histidines. These unusual situations suggest that a more accurate classification of cytochromes may be necessary. The redox potential of cytochromes with two axial histidine ligands is usually lower than that of cytochromes with one histidine and one methionine axial ligand¹². The redox potential of cytochrome *b*₅ ($E'_0 = 20$ mV)²² is lower than that of cytochrome *b*₅₆₂ ($E'_0 = 110$ mV)⁴. The completion of the cytochrome *b*₅₆₂ structure should be important for a better understanding of the structural control of the redox potentials of cytochromes, and of their evolutionary relationship.

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Dynamic model of globular protein conformations based on NMR studies in solution

BASIC PANCREATIC TRYPSIN INHIBITOR (BPTI) is a small globular protein of molecular weight 6,500 (refs 1-17). It consists of one polypeptide chain with 58 amino acid residues, including three disulphide bonds and four phenylalanines and four tyrosines as the only aromatics¹⁸. The crystal structure at 1.5 Å resolution has been extensively refined¹⁹. In the ¹H- and ¹³C-NMR (nuclear magnetic resonance) spectra, a large number of resolved resonance lines have been assigned to particular residues in the amino acid sequence^{6,8,10-16} and related to particular aspects of the molecular conformation¹⁻¹⁷. Hence, NMR spectral similarities and differences between BPTI and related proteins can on the basis of these earlier studies be interpreted in terms of structural features in well defined locations of the protein molecule. We report here the use of NMR observations to describe the globular BPTI conformation in terms of a dynamic ensemble of rapidly interconverting molecular structures. BPTI was a suitable compound for this study as its globular form is outstandingly stable in aqueous solution (Table 1)^{1,9,20}, leaving a large experimentally accessible range for studies of modified BPTI species with reduced stability. The chemically modified and homologous proteins used are characterised in the footnotes to Table 1.

Using the extensively refined X-ray structure¹⁹ and the well resolved NMR spectra it has been demonstrated that the average molecular conformations of BPTI in crystal form and in solution are, with the exception of localised regions on the protein surface^{8,17}, very similar^{1,2,14,16} and the structure type

seen in BPTI crystals prevailed also in aqueous solutions of all the proteins in Table 1^{13,21-23}. In this study the information on the solution conformations obtained from comparison with the crystal structure of BPTI was complemented by NMR measurements of the denaturation temperature, the amide proton exchange rates with the solvent in D₂O solution and the intramolecular mobility of the aromatic rings. The conclusions on the molecular dynamics are based largely on comparison of these features in the different structurally closely related proteins of Table 1.

The denaturation temperatures (see Table 1) were determined from the transition of the ¹H-NMR spectrum of the globular proteins to a random coil spectrum^{1,27}. An additional important observation was that at the denaturation temperature, the exchange between globular and denatured protein, was slow on the NMR time scale¹⁴.

The amide proton exchange rates were obtained for numerous individual protons by measurements of the decrease of the resonance intensities with time after dissolving the proteins in D₂O (ref. 27). Approximately 25 interior amide protons could readily be studied using this technique^{1,2}. Table 1 shows rate constants for the most slowly exchanging amide proton in the different proteins^{13,22,23}.

High resolution ¹H-NMR spectra provided an unambiguous criterion to determine rotational motions of the aromatic rings of phenylalanine and tyrosine about the C_β-C_γ single bond²⁷. In BPTI, three aromatic rings were found to undergo 180° flip motions about the C_β-C_γ bond²⁸ with frequencies of the order of 50-1,500 s⁻¹ at 40° and activation energies ΔG* of the order of 15 kcal mol⁻¹. One ring was immobilised over the entire accessible temperature range, with ΔG* ≈ 20 kcal mol⁻¹ at 80° and four rings were flipping too rapidly to be studied quantitatively^{3,5,9}. In Table 1 the flipping motions for two selected rings are characterised for the different proteins.

Three features of the data obtained are worthy of attention. (1) The amide proton exchange rates are outstandingly slow for globular proteins^{29,30}, with life times of the order of several years for the most slowly exchanging protons in BPTI at neutral pH and 25°C (refs 1, 2). On the other hand, local flexibility of the protein structures is implied by the rotational motions of the aromatic rings^{3,9,27,28,31}. (2) The amide proton exchange rates are correlated with the thermal stability of the globular conformation, that is, in proteins with lower denaturation tempera-

Table 1 Denaturation temperatures, amide proton exchange rates and intramolecular mobility of aromatic rings in BPTI and six related proteins

Protein*	Denaturation† T _D (°C)	NH exchange‡ k _{EX} (min ⁻¹)	Mobility of aromatic rings§			
			Tyr 35		Phe 45	
			ν (s ⁻¹)	ΔG* (kcal mol ⁻¹)	ν (s ⁻¹)	ΔG* (kcal mol ⁻¹)
BPTI	>95	2 × 10 ⁻⁷	6	16.5	30	14.2
CTI	70	6 × 10 ⁻⁵	~6	~16.5	~300	~13.0
HPI	60	5 × 10 ⁻³	6	16.5	150	13.3
R-BPTI	76	1 × 10 ⁻⁴	~6	~16.5	30	14.2
BPTI ¹	88	5 × 10 ⁻⁵	—	—	60	13.9
Des(A, R)BPTI	>65	7 × 10 ⁻⁵	—	—	60	13.9
TRAM-BPTI	>95¶	—¶	6	16.5	30	14.2

* BPTI, basic pancreatic trypsin inhibitor; HPI, isoinhibitor K from *Helix pomatia*²⁴; CTI, cow colostrum trypsin inhibitor²⁵; R-BPTI, modified BPTI obtained by reduction of the disulphide bond 14-38, with the cysteinyl residues protected by carboxamidomethylation²³; BPTI¹, modified BPTI obtained by cleavage of the peptide bond Lys 15-Ala 16 (ref. 26); Des(A, R)BPTI, modified BPTI obtained by cleavage of the peptide bond Lys 15-Ala 16 and removal of Ala 16 and Arg 17 (ref. 26); TRAM-BPTI, modified BPTI obtained by transamination of the α-amino group of Arg 1 (ref. 17).

† T_D, temperature at which at pH 5.0 equal concentrations of globular and denatured protein were observed.

‡ k_{EX}, Rate constant for the most slowly exchanging amide proton at pH 4.5 and 36°C.

§ ν, Frequency at which the rings undergo 180° flips about the C_β-C_γ bond^{3,28}, ΔG*, activation energy for these rotational motions. The data for Tyr 35 were obtained at 27°, those for Phe 45 at 4°C.

|| In these proteins, the modifications are immediately adjacent to Tyr 35; as a consequence, the Tyr 35 spectrum corresponds to the situation of rapid exchange over the entire accessible temperature range.

¶ BPTI and TRAM-BPTI were compared in different conditions. In 1M guanidinium chloride solution at pH 6.0, T_D was 75° for BPTI and 68° for TRAM-BPTI. At pH 6.0 and 55°, k_{EX} was approximately eight times larger for TRAM-BPTI than for native BPTI.

ture the amide proton exchange is faster. (3) The rotational motions of the aromatic rings are not correlated with the thermal stability or the amide proton exchange, that is, for corresponding rings similar flip frequencies prevailed in the different proteins, unless the immediate ring environment was affected by the protein modification. These observations were used to characterise the globular solution conformations of the proteins studied.

Earlier investigations of amide proton exchange in globular proteins have resulted in the suggestion that the exchange occurs by admixture of 'open' structures, O(H), to the 'closed' globular form of the molecules, C(H) (refs 29, 30). Depending on the relative rates for the closing of the protein, O(H) $\rightarrow$ C(H), and the acid/base catalysed exchange of exposed amide protons in the open forms O(H), the overall proton exchange rate k_{EX} may be governed either by an EX₁ or an EX₂ mechanism^{29,30}. For BPTI, an EX₂ mechanism was indicated by the pH dependence of k_{EX} in the range 0.5–11.0. The correlation between denaturation temperature and amide proton exchange rates for the different proteins in Table 1 further implies that the equilibrium C(H) $\rightleftharpoons$ O(H) involves global variations of the protein structure. This is also supported by the observation that, with few exceptions, the order of the relative exchange rates for the individual amide protons was the same in all the proteins studied^{1,13,17,22,23}. On the other hand, the lack of a correlation between the frequencies of the 180° flips of the aromatic rings and the denaturation temperature or the amide proton exchange rates shows that the open forms O(H), which mediate the exchange of interior amide protons, cannot be in the same class as the thermally denatured proteins. This implies that the local environment of the aromatic rings in the closed form C(H) of the proteins is also essentially preserved in the open forms O(H). Independently, admixture among the open species O(H) of molecular structures corresponding to the thermally denatured protein was excluded on the grounds that the transition from denatured to globular BPTI was slow on the NMR time scale²⁷ even at the denaturation temperature T_D (ref. 14), as a slow closing rate O(H) $\rightarrow$ C(H) would not be compatible with the observed kinetics of the amide proton exchange¹³.

From the above considerations it seems that the 'globular solution conformations' of the proteins in Table 1, and probably proteins in general, are best described as dynamic ensembles of all the structural species C(H) and O(H) involved in the amide proton exchange, where the Boltzmann law favours population of species closely related to C(H) over the population of more extremely opened forms. The data suggest that previously quoted (refs 30, 32) near-equality in several cases of ΔG^0 between the closed and open protein structures involved in amide proton exchange, C(H) and O(H), and ΔG^0 between the globular conformation and the denatured protein is coincidental. In the proteins of Table 1, structural species corresponding to the thermally denatured forms are excluded by a sizeable energy barrier from the rapid conformational equilibria which govern the exchange of interior labile protons. That the hydrophobic pockets which enclose the aromatic rings in the proteins used are apparently preserved in the open structures O(H) is compatible with the assumption that the molecular fluctuations promoting the amide proton exchange consist primarily of intramolecular translational and rotational motions of intact hydrophobic domains relative to each other. Such fluctuations would primarily open the hydrogen bonded secondary structures which link the different hydrophobic pockets¹⁹. Independently, the mobility of the aromatic rings would be correlated with the internal structural flexibility of the individual hydrophobic domains. Hydrophobic stability domains as evidenced here in one class of globular proteins might quite generally play an essential role in the architecture of protein molecules, a role which may so far have been assigned too one-sidedly to the regular secondary structures adopted by the polypeptide backbone³³. In particular, the lack of correlation of the preservation of the hydrophobic pockets surrounding the aromatic rings with the overall stability of the globular protein conformation

indicates that such hydrophobic domains might be formed at an early stage of polypeptide folding, possibly even as primary nucleation sites for the folding process.

The observed correlation between amide proton exchange rates and denaturation temperature (Table 1) may in this model be rationalised by the assumption that the energy barrier ΔG^* separating the globular conformation from the denatured forms of the protein can be overcome only by cooperative structural fluctuations resulting from overlap of different open structures O(H). With the increase of the population of states O(H) when approaching the denaturation temperature, the probability of such cooperative processes is greatly enhanced. The cooperative fluctuations would also cause destabilisation of the hydrophobic domains, thus causing a sharp transition to the denatured form.

While a thorough comparison of our results with other studies of protein flexibility must be deferred to a subsequent, more detailed paper³⁶, it seems important to point out that the many-parameter characterisation of internal motions in globular proteins by high resolution NMR provides a considerably more detailed description of interior parts of the molecules than that implied, for example, by the terms 'fluid-like' or 'continuous visco-elastic'³⁵ suggested in interpretations of theoretical model studies.

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Two contiguous conformations in a nucleic acid duplex

DNA CONFORMATIONS and properties depend on DNA sequence and on environmental conditions¹⁻³. It has been suggested that specific regulatory sites on DNA might have unique secondary conformations². Conformational transitions, and the propagation of these transitions along a DNA helix, have been proposed as mechanistic steps in RNA transcription⁴ and DNA replication^{3,5} as well as having possible regulatory roles in these functions⁶. Whether two significantly different conformations can coexist in a DNA duplex, however, has been uncertain. The *A* and *B* structural forms of DNA differ greatly in base-pair tilt, base-pair positioning and sugar ring pucker^{7,8}. Most DNA duplexes are generally considered to adopt the *B* form in solution whereas RNA duplexes and DNA · RNA hybrids have only been found to adopt *A* forms⁹⁻¹¹. We have synthesised a polynucleotide complex (Fig. 1) which has covalently linked DNA · DNA duplex and RNA · DNA hybrid tracts. NMR studies on this molecule demonstrate that the *A* and *B* conformations can coexist along a nucleic acid duplex and also that significant propagation of conformational transitions along DNA helices is unlikely.

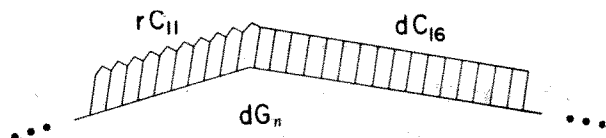


Fig. 1 A schematic diagram illustrating the coexistence of two different helical conformations in the $dG_n \cdot rC_{11}dC_{16}$ duplex.

Synthesis of the block complex, $dG_n \cdot rC_{11}dC_{16}$, was achieved as follows. rC_n and dC_n were hydrolysed to oligonucleotides by partial alkaline¹² and pancreatic DNase¹³ digestion, respectively. rC_{11} and dC_{16} oligomers were isolated from the digests using reversed-phase ion-exchange chromatography (RPC-5) (ref. 14). Alkaline phosphatase treatment removed the 3'-terminal phosphate from rC_{11} ; dC_{16} was acetylated at the terminal 3'-OH position using acetic anhydride¹⁵. rC_{11} and $dC_{16}OAc$ were mixed with dI_n and T4 DNA ligase was used to covalently link the RNA and DNA oligomers¹⁶. The product oligonucleotide, $rC_{11}dC_{16}OAc$, was isolated from both the dI_n template and the unreacted rC_{11} and $dC_{16}OAc$ substrates by RPC-5 chromatography at 65 °C. Finally, $rC_{11}dC_{16}OAc$ and dG_n ($S_{20,w} \approx 3$) were annealed to form Watson-Crick duplexes according to a previously published procedure¹⁷, which also removed the acetyl group from the 3'-OH terminus of the block oligonucleotide. Four milligrams of $dG_n \cdot rC_{11}dC_{16}$ were prepared for high resolution proton magnetic resonance studies. Physical, spectroscopic and biochemical determinations similar to those reported previously^{2,12-14} were used to characterise this nucleic acid (E.S. and R.D.W., in preparation).

In Fig. 2 the resonances in the proton NMR spectrum of $dG_n \cdot rC_{11}dC_{16}$ are compared to control $dG_n \cdot rC_{22}$ and $dG_n \cdot dC_{22}$ spectra. For $dG_n \cdot rC_{22}$, the resonance from the hydrogen-bonded G-N₁ proton is at 12.7 p.p.m. and the G-H₈ and C-H₆ protons resonate at 7.2 and 7.8 p.p.m. respectively. The positions of all these resonances are similar to the analogous resonances for $rG_n \cdot rC_n$ (see Fig. 2 legend). X-ray diffraction studies on $rG_n \cdot rC_n$ and $dG_n \cdot rC_n$ (E.S., R.D.W., A.G.W. Leslie and S. Arnott, unpublished) in polynucleotide fibres indicate that these duplexes adopt only *A* conformations.

For $dG_n \cdot dC_{22}$, on the other hand, the G-N₁ proton resonance is at 12.98 p.p.m. and both G-H₈ and C-H₆ protons resonate at 7.55 p.p.m. X-ray fibre diffraction indicates that $dG_n \cdot dC_n$ can adopt either *A* or *B* conformations¹⁸. In the conditions of high relative humidity usually extrapolated to solution studies, the *B* conformation is observed. The NMR spectrum of $dG_n \cdot dC_{22}$ supports this extrapolation since the

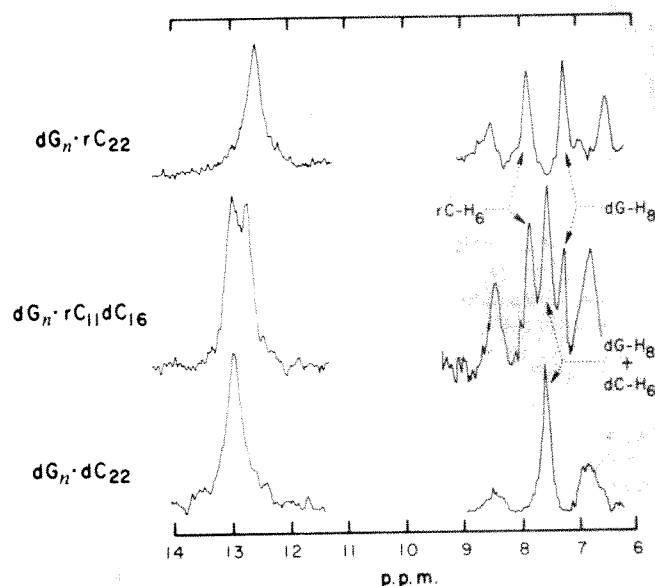


Fig. 2 A comparison of the 300 MHz NMR spectrum of $dG_n \cdot rC_{11}dC_{16}$ with the spectra of appropriate control samples. The assignments of the various resonances are indicated. Spectra were obtained using a Varian HR-300 spectrometer operated in the field sweep mode. The spectra shown were measured at 35 °C except for $dG_n \cdot rC_{22}$ which was measured at 22 °C. The low field spectra (12–14 p.p.m.) for $dG_n \cdot rC_{22}$ and for $dG_n \cdot rC_{11}dC_{16}$ are at twice the gain of the spectra for the aromatic regions (6–9 p.p.m.). For $dG_n \cdot dC_{22}$, the low field spectrum is at four times the gain of the aromatic region. NMR samples were prepared by dialysing against a dilute buffer solution and then concentrating the sample 20-fold by rotary evaporation. The final buffer concentration was about 50 mM phosphate, 0.5 mM EDTA, pH 7.0. 2–3 mg of nucleic acid was present in the final volume of 0.12 ml. For $rG_n \cdot rC_n$ at room temperature, the resonance from the hydrogen-bonded G-N₁ proton is at 12.6 p.p.m. whereas the G-H₈ and C-H₆ protons resonate at 7.27 and 7.95 p.p.m., respectively (D. Lerner, unpublished).

observed resonance positions for this complex are consistent with those calculated using ring current shift theory for stacked G · C base pairs in the *B*-DNA conformation^{19,20}. The assignment of *A* and *B* conformations in solution for $dG_n \cdot rC_{22}$ and $dG_n \cdot dC_{22}$, respectively, is in accordance with other NMR studies on oligonucleotides which show that ribonucleic acids prefer *A* forms²¹⁻²³ whereas deoxyribonucleic acids prefer *B* forms²⁴⁻²⁶.

The proton NMR spectrum of $dG_n \cdot rC_{11}dC_{16}$ shown in Fig. 2 is clearly a composite of the $dG_n \cdot rC_{22}$ and $dG_n \cdot dC_{22}$ spectra. The ratios of the resonance intensities at the various positions are consistent with the 11 : 16 hybrid to DNA duplex ratio of the complex. The obvious conclusion from this spectrum is that the two ends of the block complex adopt two different conformations. Apparently, the significant differences between the *A* conformation of the RNA · DNA hybrid end of the molecule and the *B* conformation of the DNA · DNA end can be accommodated so that the two structures coexist within a single short oligonucleotide duplex.

We recognise that the NMR spectrum presented in Fig. 2 for $dG_n \cdot rC_{11}dC_{16}$ could be interpreted as an equilibrium mixture of duplexes totally in one conformation or the other, rather than two conformations (*A* and *B*) present in a single duplex. This is unlikely since: (1) RNA · DNA hybrids have never been found to adopt *B* conformations; (2) judging from the line widths, the rate of conversion between the two conformations in equilibrium would have to be extremely slow (less than 30 s⁻¹); (3) the equilibrium constant of the system would have to be accidentally very close to 11/16 and (4) when actinomycin D, which binds to DNA duplexes but not to DNA · RNA hybrids²⁷ was added to the block polymer sample, resonances from 12.98 p.p.m. (*B* form) were selectively shifted upfield to 11.9 p.p.m. with no

effect on the intensity in the 12.7 p.p.m. (A form) peak (data not shown). This result is difficult to explain in terms of an equilibrium model. We feel these arguments amply justify our straightforward interpretation of the spectra.

The peak positions of the spectra shown in Fig. 2 for the block polymer $dG_n \cdot rC_{11}dC_{16}$ are indistinguishable from the spectra found with 3-component helix $dG_n \cdot (rC_{18} + dC_{18})$ (data not shown). Thus, any disruption of the nucleic acid helix of $dG_n \cdot rC_{11}dC_{16}$, which is created at the junction of the A and B conformations, is minimal as measured by proton NMR.

X-ray diffraction and infrared dichroism studies have indicated that $dG_n \cdot dC_n$ helices show a greater disposition to adopt A conformations than DNAs having random base sequences^{18,28}. Nevertheless, the $dG \cdot dC$ end of $dG_n \cdot rC_{11}dC_{16}$ maintains a B conformation despite the adjacent A conformation of the RNA · DNA hybrid. This finding provides clear evidence against significant propagation of secondary conformations along DNA helices, and is consistent with earlier NMR studies on $(dC_{15}dA_{15}) \cdot (dT_{15}dG_{15})$ ²⁰ and with investigations of drug binding to DNA²⁹. Propagation of these types of structural transitions in linear DNA cannot, therefore, explain either long-range genetic regulation such as seen in the arabinose operon³⁰ or some of the steps in DNA replication^{3,5}.

The presence of two conformations in a single duplex must necessitate some perturbation of the helix at the junction of the conformations. The peaks in the NMR spectrum of $dG_n \cdot rC_{11}dC_{16}$ are not sufficiently resolved to provide detailed information concerning this point. Model building, however, shows that neighbouring A and B conformations in a nucleic acid duplex can result in a 'bend', that is, a change in the direction of the duplex helix axis, with a junction involving only one base-pair (E.S., R.D.W., C. J. Alden and S. Arnott, manuscript in preparation).

This study demonstrates that two quite different conformations can exist as neighbours on a DNA helix. Thus, sequence-specific conformational sites, possibly present at genomic regulatory regions, could exist if these sequences were approximately one turn of helix (10 base pairs) in length. Shorter conformationally unique sequences might also be allowed. Prime candidates for structurally unique sites on natural DNAs would be the dA : dT sequences found in several eukaryotes^{31,32}. $dA_n \cdot dT_n$ has only been observed in the B' conformation, a variant of the classical B-DNA structure, and never in an A form³³. This behaviour may be relevant to the postulated role of dA · dT tracts in termination of RNA transcription³⁴ since RNA · DNA hybrids are apparently limited to A conformations. Other candidates for conformationally unique sites in DNA include regulatory control regions, for example the *lac* operator has been implicated to be structurally unique³⁵. These concepts have been recently reviewed¹.

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Comparison of theory to experiment for DNA thermal denaturation

THEORETICAL profiles of the thermal denaturation of random sequence DNA that were calculated by Frank-Kamenetskii and Vologodskii¹ displayed a number of narrow subtransitions. In several ways these subtransitions resembled the thermalities observed experimentally in derivative denaturation profiles^{2–7}. Lyubchenko *et al.*⁸ extended their theoretical work to include the known sequence of Φ X174 DNA and claim that not only the shape of prominent subtransitions, but also the number and denaturation temperatures are essentially the same for both calculated and observed denaturation profiles. We suggest that the agreement between current theory and experiment is not as satisfactory as has been implied, and that at least one critical parameter has been neglected in the theory and ignored in the discussions of Lyubchenko *et al.*⁸.

Thermal denaturation profiles were calculated for the double-stranded DNA of Φ X174, using the complete nucleotide sequence published by Sanger *et al.*⁹. These calculations utilised the Fixman and Frère¹⁰ modification of Poland's method¹¹, and assumed the generally acceptable values of parameters chosen by Lyubchenko *et al.*⁸ for their calculations with the same DNA sequence. As expected, our theoretical results for the whole virus DNA closely parallel those of Lyubchenko *et al.*⁸. However, we find that the theoretical denaturation profile of the *HapII*, Y1 restriction fragment of Φ X significantly departs from that published by Lyubchenko *et al.*⁸, rendering the comparison of the calculated profile to the corresponding experimental data⁴ notably less precise. We do not understand this discrepancy.

The theoretical denaturation profiles for the whole virus DNA are illustrated in Fig. 1a and b, with values of the parameters T_{AT} and T_{GC} (the average melting temperatures of the A · T and G · C base pairs within a duplex polymer), corresponding to 30 mM and 195 mM sodium ion concentrations, respectively. As noted in Fig. 1 legend, the parameters σ , α , and U are identical to those of Lyubchenko *et al.*⁸; the values for T_{AT} and T_{GC} are based on the values of Lyubchenko *et al.*⁸ (which correspond to 0.1 SSC or 19.5 mM Na^+), together with an extrapolation of $15.7^\circ C / \log [Na^+]$, which brings the midpoint of the theoretical and experimental profiles into maximum correspondence for this DNA.

Evidently, the main features of the theoretical profiles do not vary appreciably with salt concentration, except for the requisite

translation in temperature. Indeed, the small difference in shape between our theoretical profiles is largely removed if the variation of the denaturation enthalpy with salt concentration¹² is taken into account. As previously reported^{7,8}, the main features of the theoretical profiles are insensitive to moderate changes in the parameters σ and $T_{GC} - T_{AT}$, and only small changes in the profile were demonstrable when different sequence termini were used to initiate the calculation. We have also subjected this DNA sequence to a 3% random substitution of base pairs to estimate the influence of possible errors in the sequence of Sanger *et al.*⁹; no visible change in the profile resulted.

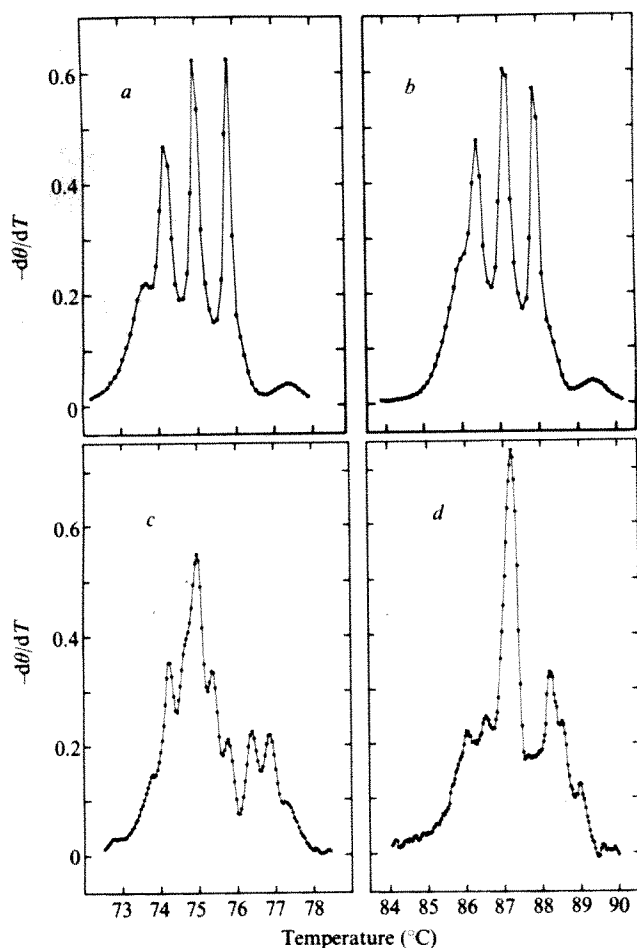


Fig. 1 *a, b*, The theoretical helix-to-coil transition profiles of duplex Φ X174 DNA. The parameters used for this calculation were $\sigma = 5 \times 10^{-5}$ (cooperativity factor), $\alpha = 3/2$ (loop entropy exponent), and $U = 8$ kcal per mol base pair (transition enthalpy). The thermal derivative of the mass fraction in coil form ($-d\theta/dT$) is plotted in (*a*) for $T_{AT} = 328.9$ K and $T_{GC} = 371.3$ K, which should correspond to 30 mM Na^+ , and in (*b*) for $T_{AT} = 341.4$ K and $T_{GC} = 383.8$ K, which should correspond to 195 mM Na^+ (SSC). *c, d*, Experimental high resolution thermal denaturation profiles of Φ X174 RF III DNA. The solution for (*c*) contains 30 mM Na^+ (29 mM Cl^- , 1 mM cacodylate, 10^{-4} M EDTA, pH 7), and that for (*d*) contains 195 mM Na^+ (150 mM Cl^- , 15 mM citrate, pH 7.2). The thermal derivative of the denatured mass fraction ($-d\theta/dT$) was calculated from the optical hyperchromicity at 270 nm using a 9-point least-squares cubic polynomial fitting routine^{2,3}. Standard deviations and fitting routine distortions are approximately the size of the illustrated points. Since the replicative form (RF-I) of Φ X174 cannot denature as a normal linear duplex because of its topological constraints, the initial DNA preparation was subjected to a single double-strand break per molecule using the endonuclease from *Pseudomonas* Bal-31 [*Altaromonas espejiana*]. The repurified RF-III molecules were then subjected to denaturation analysis. We assume that the break occurs randomly along the molecule, as is the case for supercoiled SV40 DNA (unpublished data).

For a quantitative comparison of theory and experiment we have collected high resolution thermal denaturation data on double-stranded am3 Φ X174 DNA in solutions containing 30 mM and 195 mM sodium ions (Fig. 1*c, d*, respectively). The techniques and equipment used were previously described^{2,3}. These results were obtained in conditions that closely approximate equilibrium throughout the helix-to-coil transition and should be directly comparable to the theoretical profiles of Fig. 1*a* and *b*.

Clearly, the thermalites of the two theoretical profiles bear no obvious systematic relationship to the experimental profiles. More importantly, the theory totally fails to reflect the radical changes that occur in denaturation profiles upon variation of the concentration of DNA counterion. In agreement with our previous studies², the number and mean breadth of the thermalites composing our experimental profiles appear similar although the two profiles are obviously distinctive. We must also point out that the mean breadth of the resolvable thermalites in the experimental profiles is consistent with our previous measurements² but is significantly narrower than the subtransition breadths published by Wada *et al.*⁴ for two Φ X174 restriction fragments. This difference probably arises because of our higher thermal resolution^{2,3}. A similar examination of component breadth could not be made on the data of Lyubchenko *et al.*⁸, whose experimental resolution was substantially lower. However, there is a distinct similarity between their 0.1 SSC experimental profiles and our 30 mM profile (Fig. 1*c*), and our experimental results in 0.1 SSC (not shown) are in general agreement, although our resolution clearly delineates maxima and minima where inflections appear in the data of Lyubchenko *et al.*⁸. Further, their 74–75 °C component is several times larger than the corresponding region in our results, which might reflect a bacterial DNA contamination mentioned by those authors.

It must be concluded that our understanding of the process of DNA thermal denaturation is incomplete, and it is not clear what modifications of the theory need be made. Including more sequence information as done by Wartell¹³ or distinguishing sequence doublets as proposed by Borer *et al.*¹⁴ may advance the precision of the calculation, but is not likely to provide the major theoretical revision necessary to accommodate the critical dependence of the denaturation profile upon salt concentration. Indeed, the latter observation may be taken as evidence that an electrostatic determinant differentially influences the stability of cooperative sequences, as we have previously suggested². Until the mechanism underlying this effect has been satisfactorily described, it is clear that the theory of DNA denaturation must be considered inadequate.

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matters arising

The *Q*-statistic and the diversity of floras

LAMONT ET AL.¹ accept in principal the *Q*-, or quartile, statistic for floral diversity, combining both equitability and species richness, that we proposed^{2,7} and defined as

$$Q = \frac{\frac{1}{2}S}{\log_e R_2 - \log_e R_1}$$

where *S* is the total number of species in the sample. We selected the 25% (*R*₁) and 75% (*R*₂) quantiles, the quartiles, and evaluated the characteristic slope of the cumulative species abundance curve over this range because, from our experience of insect catches³, a considerable proportion of the species may be represented by singletons. We would expect this quartile statistic to be less affected by sample size than that based on the 10% and 90% quantiles proposed by Lamont *et al.*¹. However, we are more concerned about their justification for the statistic and the results thereby obtained.

Although their characteristic slope, like the *Q*-statistic, may be equated with the reciprocal of the slope of the rank-abundance plot (Fig. 1), standard regression methods are not applicable in the way Lamont *et al.* suggest. Because successive points are very highly correlated, sampling errors will be considerably underestimated, and the method breaks down for discrete data, like those quoted for Malaysian rain forest⁴, in which a large proportion of species are represented by one or two individuals. Our own technique is to fit a stabilising model to the species frequency distribution, rather than to the rank-abundance plot, by weighted least squares⁵. The statistic may then be derived from the parameters of the model (for the log-normal $Q = 0.371 S/\sigma$ and for the log-series $Q = \alpha$) and this method will be highly efficient and applicable for both continuous and discrete data.

Lamont *et al.*'s failure to fit a model¹, in particular the log-normal, seems to discourage them. However, comparison of our Fig. 1 with theirs suggests that the fault lies, not in the models but in their fitting procedures. In spite of the floral richness of their site the abundance distribution does not seem unusual. Further, their curve-fitting tests, based on comparison of the residual sums of squares with the percentage points of a χ^2 distribution (their Table 1), are inappropriate because the size of these residuals will depend on the scaling used.

Using the more appropriate techniques now available in the MLP program⁶ we have fitted the log-normal and gamma³ models (the simpler log-series being unsuitable for continuous data¹) to the heathland data they produced. The fit of both models is good, the gamma slightly the better of the two (Table 1), and, while the two models predict different numbers for the rarest and commonest species, the *Q*-statistics for the midrange species are similar and close to the empirical value obtained by evaluating the quartiles *R*₁ and *R*₂ directly from the sample values (Table 2).

Lamont *et al.*¹ state that the diversity of their heathland sample is "as high if not higher than those for the most diverse communities previously recorded, including the richest of tropical rain-forests". But, when all the diversity indices are evaluated for the data quoted by Lamont *et al.* for Malaysian tropical rain forest^{1,4}, they are found to be consistently higher than those for Western Australian heathland by a factor of between two and three (Table 2).

Table 1 Fit of log-normal and gamma models to species frequency data from the Western-Australian heathland site

Importance value class	No. species observed	No. expected log-normal	No. expected gamma
<0.05	14	10.0	17.9
0.05–0.1	8	12.2	9.1
0.1–0.2	20	20.0	13.5
0.2–0.5	35	36.5	27.0
0.5–1.0	23	29.8	28.2
1.0–2.0	30	25.8	32.1
2.0–5.0	30	22.7	33.7
5.0–10.0	10	8.8	9.3
>10.0	2	6.2	1.1

Parameter estimates:

log-normal ($\chi^2_6 = 11.7, P > 0.05$)

$\mu = -0.53, \sigma = 1.57$

Gamma ($\chi^2_6 = 8.2, P > 0.05$)

$m = 1.51, k = 0.60$

The chi-squared goodness of fit test relies on the assumption that number of species observed in each class is a Poisson variable.

Table 2 Values of indices of diversity for Malaysian rain forest and Western Australian heathland

Index	Formula	Tropical rain forest Malaya ⁴	Heathland site Western Australia ¹	Ratio forest/heath
Simpson's (inverse)	$\frac{1}{\sum_i p_i^2}$	84.2	32.7	2.57
Shannon-Wiener (exponent)	$\exp\left(-\sum_i p_i \log p_i\right)$	149.1	69.4	2.15
<i>Q</i> -statistic	$\frac{\frac{1}{2}S}{\log(R_2/R_1)}$	104.6	38.2	2.74
	log-normal	105.9	40.6	2.61
	gamma	105.6	38.6	2.74
	log-series (α)	114.2	28.0*	
Whittaker's	$\frac{S}{4[(\sum_i (\log p_i - \log \bar{p})^2/S)]^{\frac{1}{2}}}$	77.1	28.3	2.72
log-cycle	$\frac{S}{\log p_1 - \log p_s}$	80.7	21.2 (24.4)†	3.81 (3.31)†

All indices given by Lamont *et al.* are included with the exception of Gleason's index whose value is directly related to population density. *S* is the total no. of species in the sample, *p_i* the relative frequency of the *i*th most frequently occurring species, $\log \bar{p} = (\sum \log p_i)/S$, *R*₁ and *R*₂ the quartiles of the frequency distribution. The logarithms are taken to base *e*. The *Q*-statistic is derived directly from the data and from the parameters of three fitted models.

* The log-series diversity index α is not directly comparable with other indices for the heathland site as it is calculated from the species numerical abundances not their IVI importance values.

† Bracketed figures are obtained by omitting single rarest species. This operation has a negligible effect on other indices.

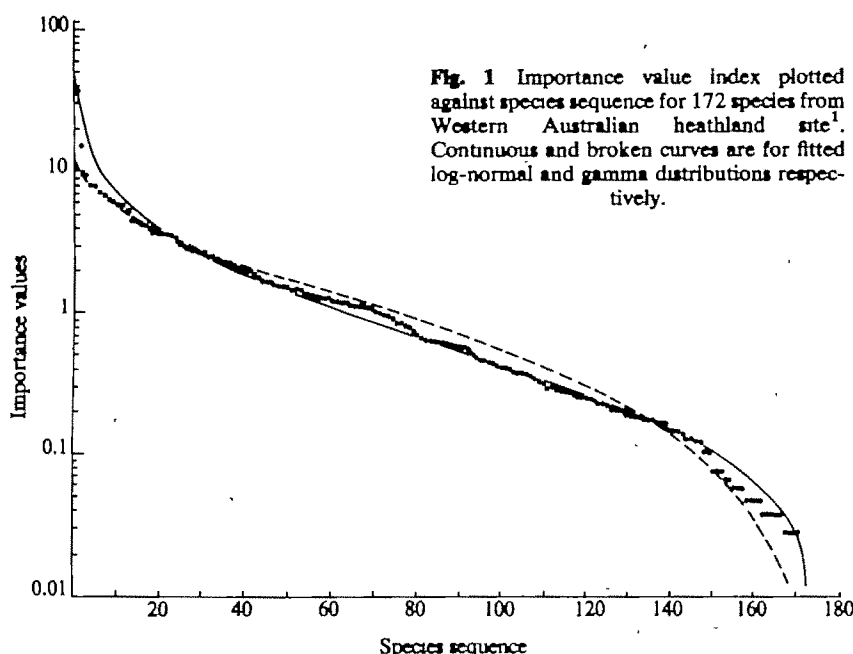


Fig. 1 Importance value index plotted against species sequence for 172 species from Western Australian heathland site¹. Continuous and broken curves are for fitted log-normal and gamma distributions respectively.

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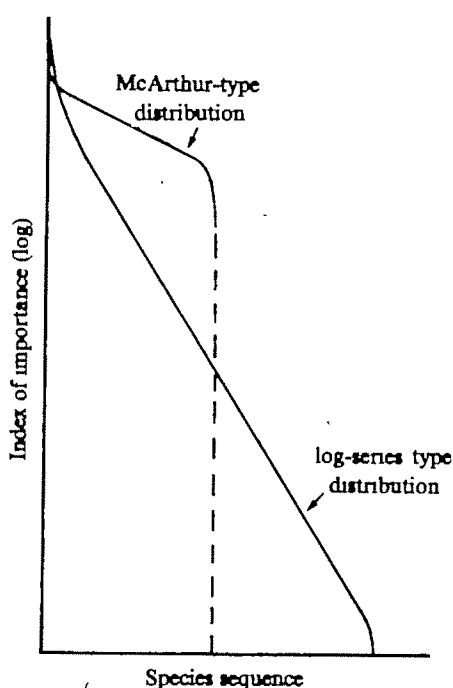
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LAMONT ET AL. REPLY—Use of the best-fit ('characteristic') slope of the importance-value curve as a measure of diversity has intuitive appeal. The problem in species rich samples, however, is that nonlinear models generally fit the data better than linear ones. In view of this, use of the variance might prove an acceptable alternative. We maintain that both these measures are indices of evenness and not of total diversity, such as Shannon's which confounds both evenness and richness (Fig. 1).

Comparison of our heathland data against results for Preston's classic curve-fitting procedure for the log-normal distribution^{1,2}, using the χ^2 test favoured by Kempton and Taylor³, confirms our previous conclusion of their dissimilarity⁴. Using the more recently devised methods

Fig. 1 The relationship between evenness and total diversity: one community (for example representing a McArthur distribution) has half the slope (twice the evenness in terms of number of species per unit importance), but only half the number of species (half the richness) as the other (for example representing a log-series distribution). Only when these figures are multiplied do we have an indication of total diversity—in this case they should be the same.

of estimating log-normal and gamma parameters, adopted by Kempton and Taylor, we now see that the difference becomes nonsignificant. Because lumping may mask variation in the original data, particularly in the dominant-species region (a limitation of the variance approach to evenness) it is desirable to compare the log-normal curve against the observed curve. In our original paper⁴, the scaling was fixed so the χ^2 values for the fitted curves gave some indication of their relative goodness of fit, but the significance levels should be ignored. We relied, however, on the more appropriate one-sample, Kolmogorov-Smirnov test⁵. The observed and expected importances for the log-normal fit by Kempton and Taylor above were significantly different at the 0.1% level,



and for the gamma fit at the 5% level. The latter is worse than the log-series, but better than the (linear) exponential⁴.

We agree with Kempton and Taylor that the gamma index (S/σ^2) is the best variance measure of evenness, even though it does not fit all data and is not synonymous with the best fit slope. It should not be confused with the quartile index which is an inferior slope measure³. The exponential curve remains the best measure of slope, though removal of the dominant species is sometimes required to meet the requirements of the Kolmogorov-Smirnov test, with minimal change in slope. We now consider removal of up to 10% from each tail (our original characteristic slope⁴), let alone 25% (quartile), is unwarranted.

We made it clear⁴ that William's index, based only on number of individuals (largely a function of life form), for the Malaysian rain forest was much higher than for the Western Australian heathland. We are therefore not surprised that data we considered of little relevance to community diversity (except in providing species richness/number of individuals), and incompatible with ours, continues to show a higher diversity when expressed in terms of other indices. In addition, we point out that the rain forest site was not physiographically uniform⁶, it is difficult to identify communities in this rain forest anyway⁷, the method of survey ensured high evenness: specimens had to be tall trees exceeding 30 cm in diameter but no reference was made to their size, and the sample was nearly 450 times the area of ours and 20 times its height. Had small plants been included and more acceptable measures of importance been used it is certain that the vast increase in range of values would have greatly reduced evenness. Such inclusions would no doubt have increased the slope of the species-area curve of the rain forest above that of the heathland, but the extensive experience of one of us in related Dipterocarp rain forests indicates that ten 50 m² samples which contained 150 species, let alone 172, would be most unlikely.

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Renewable energy sources and storage

LEICESTER, NEWMAN AND WRIGHT¹ claim to have invalidated Ryle's² hypothesis that windpower could make a substantial and economical contribution to Britain's electricity supply, once short-term (150-h) thermal storage is provided at places of end-use. The counter-argument of Leicester *et al.*¹ seems to be based on two principal observations. (1) Plots of average heat demand against mean windpower output, which show that the standard deviation of windpower output is large and decreases only slowly with storage capacity. And (2), their "extreme example" for which the mean wind energy available over the period 1 February–31 March 1975 was about 54% of the long-term average wind energy. Unfortunately, neither of these observations provides a test of Ryle's hypothesis.

Observation (1) confirms the well-known fact that windpower is very variable. However, since the probability distribution of windpower is highly skewed in general, it is not particularly useful to quote standard deviations. More importantly, the scatter diagrams do not necessarily provide useful information on the crucial problem of storage content. The proposition by Leicester *et al.* that their interpretation of Ryle's thesis implies that the points in their Fig. 2b should be grouped around the straight line through the origin of slope unity, is false. The grouping is sufficient but not necessary. Because windpower is proportional to the cube of windspeed, the store can be filled quickly but will be emptied slowly.

A simple example brings out this inadequacy of the scatter diagrams. If the daily output is constant at 1 unit, which seems to be a reasonable first approximation to the data presented, then 11 days' successive input of $\frac{1}{2}$ unit followed by a day with an input of 6 units keeps the store non-empty at all times, given that it was initially full. Yet the running 6-day average inputs are $\frac{1}{2}$ for half the time and $1\frac{1}{2}$ for the rest, giving a wide scatter.

In contrast to their scatter diagram (Fig. 2) which is not helpful, quantitative information on windpower reliability is obtained from plots of power availability or capacity factor expressed as a function of storage capacity^{3–5}.

Observation (2) merely reiterates the well-recognised point that windpower plus short-term storage alone cannot satisfy power demand with a probability of unity. However, Ryle² does not assume that there are no conventional sources of power feeding the grid. We suggest that some of the real questions of quantitative significance raised by Ryle's work are as follows. First, what is the probability of low-wind periods of a given length? Second, how much conventional (that is,

non-renewable) baseload, intermediate load and peakload capacity must be retained in a grid fed by windpower to ensure that low-wind periods can be covered with a probability close to unity? Third, what are the additional economic costs and benefits of such a hybrid grid? For example, the costs of extra start-ups of conventional power plants during low wind periods and the losses of unutilised windpower when storages are full; the benefit in replacing new or retired fossil-fuel and nuclear plants with windpower or other renewable sources of energy; fuel-saving benefits when de-controlled as well as controlled prices are considered for fossil fuels. Finally, by how much are the answers to the second and third questions changed when a more flexible method of load switching and pricing is introduced? Such an automatic rationing system is technically feasible and could mean that instead of freezing over the period 7–16 February 1975, CEBG all-electric householders would have experienced simply a fall in home temperatures of (say) 5°C over the longer period 2–16 February. For the case of central or substation storage, we have found that a user strategy in which part of the windpower output contributes to the immediate demand and part is proportional to the fraction of energy in storage, yields a substantial increase in the availability of windpower, compared with the strategy of simply following demand (M.D. and K. Malafant, in preparation).

Leicester *et al.*¹ do not attempt to modify user demand, nor consider probabilities of extreme events, nor hybrid systems, nor economics of any kind. Their interpretation of their scatter diagrams is based on a false proposition. It is, therefore, difficult to understand the basis of their assertion that Ryle is "probably incorrect" on relative economics and that "sufficient work has already been done to demonstrate that 150-h storage is quite inadequate to smooth the output of wind generators".

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LEICESTER ET AL. REPLY—Since our earlier work we have extended our analysis to determine the amount of storage that would have been needed to match heating demand to windpower output throughout the period 1 October 1974 to 31 March 1975, assuming an energy

balance over the period as a whole. We have done this by calculating and then adding together the maximum cumulative surplus and deficit of windpower output relative to heating consumption over the 6 months. The storage requirement turns out to be 1,124 h, thus substantiating our contention that 150-h storage is quite insufficient.

We have also calculated the normalised averages of heating demand and windpower output for 1,124-h periods within the 6 months and find that the former extend from 0.9 to 1.04 and the latter from 0.4 to 1.73. The standard deviation of the heating demand is now 0.028 and that of the wind energy is 0.394, which are about half the values with 150-h storage. Although these results support Diesendorf and Westcott's argument that zero scatter is not a necessary condition of sufficiency of storage, they do show that with sufficient storage the scatter is very much smaller than for 150-h storage as shown in our Fig. 2b.

Diesendorf and Westcott raise a number of other questions which, we agree, are relevant in a wider context. However, there is clearly a difference in view between us on what Ryle has proposed. Our understanding of his concept is that, he contends that, by controlling the electrical input to storage for space heating and hot water supply by remote switching in accordance with the availability of windpower output, the heating demand can be fully and reliably satisfied without recourse to supplies from other generating plant. We believe that our analysis shows conclusively that 150-h storage is not sufficient to support Ryle's contention, but this does not mean that the use of storage with renewable energy sources is unimportant. Indeed, while it was not the purpose of our article to do more than examine the storage proposition as formulated by Ryle, we are nevertheless continuing to study the question of integrating large amounts of windpower into the CEBG's system and this will include consideration of questions (1), (2) and (3) posed by Diesendorf and Westcott.

With regard to their fourth question, a policy of selective rationing of electricity for heating is not feasible since consumers could not be denied freedom to resort to electrical supplementary heating at times of storage deficiency in cold spells. Such use would inevitably occur and the consequent peak load on the supply system could be close to what it would have been without windpower generation with the result that the latter would have minimal firm capacity.

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reviews

Understanding speciation

Godfrey M. Hewitt

Modes of Speciation. By M. J. D. White. Pp.455. (Freeman: Reading, 1978.) £18.50.

SPECIATION involves the splitting of a single evolutionary lineage into two or more genetically independent ones. This process of cladogenesis is one of the twin axes of organic evolution, the other being the process of anagenesis which involves the phyletic change of an evolutionary lineage through geological time. In the *Origin of Species by Means of Natural Selection* (1859) Charles Darwin dealt with both of these fundamental processes, although perhaps understandably at that time he was more concerned with incontrovertibly establishing anagenesis ("descent with modifications") than with discussing the mechanisms of cladogenesis ("the origin of species"). Nevertheless in chapter four a few short passages reveal that he considered both disruptive selection to fit distinct niches and geographic isolation on islands as important mechanisms of evolutionary divergence. In fact Alfred Russell Wallace (1901) considered the process of speciation more closely.

The 'Neo-Darwinian Synthesis' of evolutionary theory was made some thirty years ago and continued to emphasise phyletic change. However, geographical isolation was now considered to be of prime importance in speciation and by some as a necessary pre-condition. Consequently, nearly all books on evolution devote only a chapter or so to cladogenesis. This remains so in the recent stimulating text *Evolution* by Dobzhansky, Ayala, Stebbins and Valentine (1977), and Verne Grant's thoughtfully integrated *Organismic Evolution* (1977) devotes one of eight major sections to speciation. Ernst Mayr gives the topic major prominence in his *Populations, Species and Evolution* (1970), but this book considers only animals and like its parent volume *Animal Species and Evolution* (1963) vigorously favours geographical isolation. Grant's *Plant Speciation* (1971) is the only book to deal specifically with cladogenesis, but as the title indicates it is restricted to plants.

Against this background Michael White has decided to produce a "comparative study of speciation in relation to the population structure and genetic

architecture of living organisms". He deals with both animals and plants and considers a wide range of possible modes of speciation. This book may thus be considered as the first comprehensive treatment of the origin of species.

It is also a most timely publication. There has been an accumulation of pertinent studies over the past twenty years, the most striking discovery being the considerable level of genetic variation revealed by gel electrophoresis of proteins. And there have also been substantial advances in the less well publicised fields of chromosome variation and behavioural isolation. Some of these investigations have raised nagging doubts as to whether an extended period of geographical isolation is a necessary prerequisite for the production of genetic isolation and speciation. This growing concern can be sensed in the later texts referred to above and in a number of recent papers and reviews. As White himself recognises, "the time therefore seems ripe for a reconsideration of the whole question of the mechanisms of speciation . . . It is probable that a number of modes of speciation exist, and that the simple antithesis of allopatric and sympatric modes is a false one, since most of these involve a geographic component of some kind".

White admits in the preface that "the organisation of the book presented some major difficulties. Many controversial topics could have been discussed equally well in any of several chapters". Nevertheless, the structure which he has chosen to deal with this immense subject proves a successful vehicle to convey his message. Furthermore, the book is very readable, usefully illustrated and well produced. It contains seventy-six pages of references alone and though most of them are recent, it provides a valuable link with the older literature.

Chapter one is a lively introduction; in it he is at pains to convince doubters that species and speciation are identifiable concepts and worthy of study. This leads to a consideration of isolating mechanisms, the genetic processes involved in evolution and the type of data required for a full study of each case of speciation. He is unhappy with previous classifications of models of

speciation and proposes seven models that span the range from pure allopatry to pure sympatry. These models are then dealt with in detail in later chapters, as are polyploidy and parthenogenesis.

Before this exposition the stage is set by a chapter on the genic structure of species and a chapter on chromosomal differences between species. The former provides a balanced and pertinent account of the mushrooming data on electrophoretically detected allelic differences and the questions raised by this. Perhaps the most important conclusion is that related species may differ very little in their allozymic constitution. The massive chapter on chromosomal differences is reminiscent of the author's encyclopaedic work *Animal Cytology and Evolution* (1973). With such a vast array of information at his command the initial heading of sections certainly aids the reader, and it is a pity that this is discontinued after the section on *Drosophila*. It is his expressed intention to advocate a much larger role for chromosomal rearrangements in speciation than in earlier texts and he certainly hammers home the portentous fact that very few, if any, closely related species are chromosomally identical. From this he concludes that these chromosome changes cause speciation, but does not present fully an argument to justify this. Nevertheless, the association of chromosomal difference with speciation requires an explanation.

The allopatric models of speciation in which genetic isolation is acquired under complete geographical isolation are examined in chapter four. Several clear cases are discussed and a number previously claimed as examples are critically reassessed. These necessarily include the Galapagos Islands, the Hawaiian *Drosophilidae*, the land snails of the Society Islands and Caribbean and the species flocks of ancient lakes, as well as lesser known work in frogs, locusts and lemmings. Character displacement and reinforcement of species-isolating mechanisms are briefly treated at the end of the chapter; these contested subjects are perhaps worthy of more fundamental consideration.

Clinal and area-effect models are considered next. These propose the segregation of distinct species out of

geographical races that are spatially continuous. The thrust of this chapter depends firstly on Bryan Clarke's (1968) model for which there is suggestive evidence and secondly on the reinforcement of hybrid zones over which White is perhaps prematurely enthusiastic. However, these models are as yet relatively under-explored, and he is right to promote interest in them.

The chromosomal modes of speciation are considered seriously in the sixth chapter to account for the widespread occurrence of karyotypic differences between sister species. Harlan Lewis' pioneer work with *Clarkia* argues powerfully for saltational speciation as a form of microallopatry involving hybrid chromosomal sterility, and the bulk of the chapter describes cases that White considers to support his own stasipatric model. This latter is essentially a clinal model driven by hybrid chromosomal sterility. Some authors will possibly be surprised to find their data so interpreted, particularly when they themselves have invoked geographical isolation. Both this chapter and the last are concerned with what Murray (1972) has called parapatric speciation and further investigation will clarify our concepts.

The chapter on sympatric models of speciation is well written and the concepts are readily grasped. This is a result in part at least of the resurgence of interest in the early 1960's through studies such as those of Maynard-Smith and Thoday and Gibson on disruptive selection. The strength of the case rests with the evidence from host-specific insects, particularly that of Bush (1974) on *Rhagoletis*. White makes a good argument for sympatric speciation on small oceanic islands with many species and also for allochronic speciation.

Following a neat review of speciation by polyploidy, White considers it to be fairly insignificant in most organisms, excepting certain groups of plants such as ferns and grasses where it may perhaps have inhibited speciation by other methods.

Chapter nine extends the species concept to include isolation by asexual reproduction and we are treated to an exposition of one of White's pet subjects. He demonstrates the hybrid origin of many thelytokous species, but one wonders if heterosis of the hybrid is absolutely necessary to explain their origin. In the invertebrates at least thelytoky is a sporadic development and he emphasises that though much has been written about the vast agamic complexes in plants there are very few of these. His consideration of the currently engaging question, "What biological use is sex?" is not complete but certainly points the way, and he advocates examination of the genetic architecture of thelytokous species and

their bisexual relatives as presently more necessary than model building.

I found the last chapter (Conclusions) the most enjoyable. It contains all sorts of evidence, concepts, ideas and questions that arise from the preceding survey. One might argue that some of the points on behaviour, sex, hybridisation and reinforcement should have been considered before the models, but one must break into the cycle of scientific enquiry somewhere and this approach is certainly realistic and stimulating.

A comprehensive study of speciation

Automated chess

The Machine Plays Chess. By A. G. Bell. Pp. 114. (Pergamon: Oxford, New York and Sydney, 1978.) Hardback £5; paperback £3.

It is one of the rarer pleasures of a book reviewer to receive a text which both entertains and informs him simultaneously. This book was the source of one such pleasure.

The text commences with an excellent commentary on the principal stages in the historical development of chess automation, going back through the pre-electronic "paper machines" of the early twentieth century, the ideas of Babbage and Turing, and the famous "Turk" pseudo-automaton of the eighteenth century.

The main part of the book deals with the situation in recent years, and

requires the use of a wide range of biological studies. This text will no doubt provoke specialists in many areas, but there are so few people capable of giving us such a fine prospect. This personal view should be read by every serious biologist, for Michael White has succeeded in producing a most readable, comprehensive and stimulating thrust towards understanding speciation—may it bear fruit.

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succeeds admirably in communicating an insight into the 'thought processes' of computer programs (and their authors). The selection of example games clearly shows the super-human capabilities of the computer in tactical calculation, and with equal clarity exposes the perpetual undercurrent of planlessness which is the trademark of all automata. A sensible appendix supplements the main text with a simple explanation of the special techniques used in move selection and evaluation.

This is a book which can be recommended as almost essential for those interested in machine intelligence, would be entertaining for chessplayers of all standards, and provides an excellent introduction for those with experience of neither.

N. J. Holloway

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Neural mechanisms of memory

Elements of the Behavioural Code. By Francis V. DeFeudis and P.A.F. DeFeudis. Pp. 397. (Academic; New York, London and San Francisco, 1977.) £15; \$29.35.

WHAT progress is being made toward the understanding of neural mechanisms of memory in terms of biochemical and anatomical changes in the brain? This seems to be the overall problem set by DeFeudis and DeFeudis. Their main effort in this book has been devoted to surveying changes observed in the nervous system as a consequence of exposing experimental animals to enriched or impoverished environments, or giving them training, or using physiological and/or pharmacological techniques to alter the functional load imposed on the nervous system. Proceeding generally from broader to finer effects,

the authors describe changes found in the gross anatomy of the nervous system, in cellular and subcellular morphology, in neural macromolecules, in energy metabolism, and in presumed neurotransmitters and some other small molecules. This survey is based on an impressive list of over 1800 references, about three-fifths of them from the 1970s and including many citations from 1976 when the manuscript was completed. Following the 89-page reference list are a 29-page subject index and a 25-page author index. The selection and compilation of this material and rendering it accessible through the detailed indexes are sure to be of great help to investigators and advanced students in all the disciplines that are contributing to the study of the biology of memory.

Given the magnitude of the task, it is understandable that DeFeudis and DeFeudis have been somewhat less successful in describing and interpreting the varied and rapidly accumulating material of this field than they

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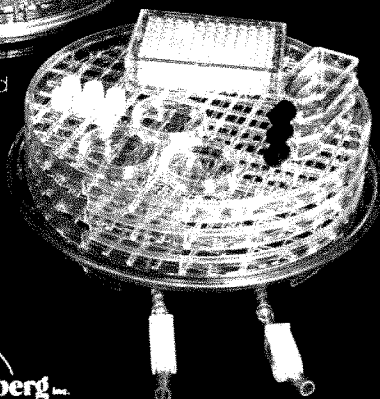
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ASA Monograph**Sex and Age as Principles of Social Differentiation**

edited by J. S. La Fontaine

September/October 1978, viii + 188pp., £6.20/\$12.75 0.12.433050.9

This book presents a collection of papers designed to focus attention on a neglected topic: the nature of ascriptive roles. Two of the most common ways of establishing social differentiation are by age and by sex, and although the two principles have usually been discussed separately, they are similar since each is a cultural transformation of a natural process of human physiology — ageing, and reproduction. Very often, too, the principles are found in conjunction, and the relationship between the sexes may depend on the existence of a ritualised distinction between mature and immature.

Developmental Dysphasia

edited by Maria A. Wyke

September/October 1978, xli + 180pp., £7.80/\$16.15 0.12.766950.7

Developmental dysphasia has attracted considerable attention for a number of years. The problem has engaged the interest of scientists from very different fields, and many attempts have been made to unravel the essential nature of the disability. For a long time the deficits of children with developmental dysphasia had been merely observed and recorded, but lately there has been an increasing number of investigations which have viewed developmental dysphasia in the light of more rigorous experimental procedures. This book presents an up to date account of the work carried out in the field of developmental dysphasia.

Adverse Response to Intravenous Drugs

edited by J. Watkins and A. Milford Ward

September/October 1978, x + 188pp., £8.20/\$17.00 0.12.737850.2*

In spite of its numerous advantages to the clinician, the intravenous route for drug administration presents certain difficulties of adverse response, with implications in many areas of medicine, notably in anaesthetics and blood plasma-substitute technology. For the most part the reactions are histaminoid or anaphylactoid, but it is clear that genetic, physiological and psychological factors also play a part in generating the adverse response. The precise nature of this response is, however, far from clear, and this book will help to throw light on the issue. Most of the papers in this volume were first presented at a Symposium held at Sheffield University Medical School in June 1977.

[Published in New York by Grune & Stratton Inc. ISBN 0.8080.1098.1]

Inorganic Polymers

N. H. Ray

September/October 1978, xli + 174pp., £8.40/\$17.35 0.12.583550.7

There is a need to develop new inorganic materials to supplement and replace synthetic organic polymers which are derived from the diminishing fossil carbon resources of the earth. With this in mind, this book provides a comprehensive survey of inorganic polymers from the viewpoint of a materials scientist, with particular emphasis on their structures and properties and the relationships between the two. In doing so it brings together data from a variety of sources not often consulted by polymer scientists, such as mineralogy, glass technology and synthetic chemistry.

ISAM '77**Proceedings of the Second International Symposium of Ambulatory Monitoring**

edited by F. D. Stott, E. B. Raftery, P. Sleight and L. Goulding

September/October 1978, xviii + 332pp., £10.50/\$21.75 0.12.672350.8

Ambulatory monitoring deals with the collection and analysis of physiological data from subjects who are free to carry out normal activities, whilst the data is being recorded. In principle, it is not a new technique; electro-cardiographic monitoring, using portable equipment, has been employed for nearly twenty years as a tool in clinical cardiology and for research. At the first symposium, ISAM '75, this was the major field of interest represented, but ambulatory monitoring of blood pressure was also an important topic. Both these fields are well represented again in this, the 2nd Symposium, but in addition other varied applications in electro-encephalography and in physical rehabilitation medicine have increased in importance.

Language, Thought and Culture: Advances in the Study of Cognition**The Cultural Definition of Political Response**

Lineal Destiny Among the Luo

David Parkin

September/October 1978, xxli + 348pp., £14.80/\$30.65 0.12.545650.6

Cultural change is not just a straightforward response to external pressures: the culture itself, as a powerful and autonomous system of ideas, plays a large part in shaping people's internal adaptation to new circumstances. Recent far-reaching changes — educational expansion, increasing wage dependency, population growth and urban unemployment — have all had their impact on the populations of Africa, but underlying cultural differences have persisted throughout. It would be misleading, therefore, to think of ethnicity as a mere 'false consciousness', without any bearing on the destiny of a people. The Luo of Kenya, who are the main subject of this investigation into the nature of cultural change and its semantic aspects, are no exception to the rule.

The Environmental History of the Near and Middle East

Since the Last Ice Age

edited by William C. Brice

September/October 1978, xx + 364pp., £16.00/\$33.15 0.12.133850.9

The landscapes between the Mediterranean and the Valley of the Indus have undergone many alterations since the climax of the last Ice Age, some 20,000 years ago: lakes have filled and dried out, rivers have shifted their courses and deserts created, forests have receded and advanced. These changes, which manifestly affected the inhabitants and their ways of life, are of particular historical moment as they coincided with crucial early experiments in the arts of agriculture and domestication; of irrigation, architecture, metallurgy, commerce, and the building of cities. This is a collection of recent observations on the nature and extent of these changes of environment, by scholars from eight countries and several disciplines.

Action, Gesture and Symbol: The Emergence of Language

edited by Andrew Lock

October/November 1978, xiv + 588pp., £22.00/\$45.50 0.12.454050.3

How does a child move from the early, pre-speech types of communication to a fully-developed use of language? This collection of essays puts the challenge: is there a developmental continuity between preverbal and verbal levels of communication; is the latter predicated on the former? The book suggests that the infant's early interaction with the social milieu lays the foundation for language, and pursues this view in five sections, each exploring a particular facet of the topic. Theoretical problems regarding the continuity of communicative systems are posed, and the evolutionary question, early mother-infant interaction, and the transition from gesture into language symbols are explored. Finally, the role of social processes in the creation of linguistic abilities is considered.

The Endocrine Hypothalamus

edited by S. L. Jeffcoate and J. S. M. Hutchinson

October/November 1978, xviii + 546pp., £23.00/\$47.50 0.12.382150.9

Recent research on the hypothalamus has led to several important endocrinal discoveries and redefinitions, particularly concerning the chemistry and functions of the hormonal peptides. This book is a much-needed, balanced critical review of the subject, written by an international panel of authors, all of whom are actively engaged in their particular subject. The first half of the text deals with general aspects of the endocrine hypothalamus; anatomy, hormones, methods of investigation and control mechanism. This is followed by more detailed chapters on the separate hypophysiotrophic functions; control of thyroid-stimulating hormone, reprogrowth hormone, prolactin, corticotrophin and melanocyte-stimulating hormone.

The Prehistory and Palaeogeography of the Great Indian Desert

Bridget Allchin, Andrew Goudie and Karunakara Hegde

March 1978, xx + 370pp., £25.00/\$48.90 0.12.050450.2

This is a study of the relationship between Stone Age cultures and changing environments in the Indian sub-continent during the latter part of the Pleistocene and Early Holocene. The authors have sought evidence of past climates differing substantially from that of the present time, and evidence of the presence of man noting in particular where the two were associated. Their field work in the desert and dry zone on its southeastern margins and in the Indus Valley showed that fluctuations in the extent of the Thar or Great Indian Desert and in the intensity of arid conditions in the region had provided a framework for environmental change.

The Systematics Association Special Volume No. 10

Modern Approaches to the Taxonomy of Red and Brown Algae

Proceedings of an International Symposium held at the Polytechnic of North London

edited by D. E. G. Irvine and J. H. Price

September/October 1978, xii + 486pp., £26.20/\$54.25 0.12.374050.9

The red and brown algae—mainly multi-cellular marine benthic organisms—offer challenging problems to the taxonomist. They are often difficult to study in the laboratory; comparatively simple in structure; often highly 'plastic' in form. The papers in this book present the proceedings of an International Symposium which was organised to bring together workers on widely different aspects of the red and brown algae; to evaluate the taxonomic potential of new data emerging from their researches; and to encourage interdisciplinary approaches. The papers provide a valuable contribution to algal taxonomy in themselves, while also indicating promising lines for future taxonomic research.

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were in compiling the references. The text itself is rather brief in relation to the apparatus of references and indexes; 248 pages of text, tables and figures could hardly be expected to describe the references cited, let alone discuss them to any degree of thoroughness. Many important studies are given only a few sentences, whereas others are mentioned in a phrase or are lumped with other related papers in an overall characterisation. There is not much critical discussion of experiments or conclusions offered by investigators, although the authors occasionally point out discrepancies among reports and suggest some factors that complicate research and interpretation.

The title and prologue of the book may set up too high expectations by indicating that "... a theory for the 'Behavioral Code' is provided ...". Probably the emphasis should be placed on the word "elements", and perhaps "ingredients" would have been a more appropriate term. Table 80 lists as "Elements of the Behavioral Code" items as diverse as environmental stimulation, initiation of nerve impulses and transmitter release, effects on rates of synthesis of cyclic nucleotides, changes in synaptic efficiency, and

changes in learning, memory, and adaptive behaviour. Elsewhere in the book we are told that both short-term and long-term memory are elements and that "... neurotransmitter suspects [that is, presumed neurotransmitters] are integral elements of the Behavioral Code."

Apparently, although no definition is given, the Code when completed will include all of the events and processes that must intervene between initial capture of sensory information and storage of memory. Even the retrieval of memories should be included, although this book reflects most of the work in the field by not even mentioning retrieval. DeFeudis and DeFeudis believe, as do many workers in this field, that storage of memory requires a complex series of processes rather than depending upon some one key process. Their book is a substantial contribution towards the eventual formulation of a comprehensive theory of the neural mechanisms of learning and memory.

Mark R. Rosenzweig

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Vertebrate visual system physiology

Handbook of Sensory Physiology. Vol. VII/5: The Visual System in Vertebrates. Edited by F. Crescitelli. (Springer: Berlin, Heidelberg and New York, 1978.) DM 340; \$149.60

THIS volume contains contributions by Crescitelli, Dvorak, Eder, Granda, Hamasaki, Holmberg, Hughes, Locket, McFarland, Meyer, Muntz, Munz, Olson and Reyer. The subject matter is concerned with the morphology, optics, pigments, physiology and adaptive radiation of the eye in different vertebrate classes. The chapters are comfortably short; most are under one hundred pages in length.

This volume is both necessary and important as a supplement to the earlier volumes in the *Handbook*. It is a necessary text for those libraries which deal with visual science, whether in its medical, biological or behavioural aspects, and is important in providing a source-book which is reasonably comprehensive and broad in outlook. It is occasionally suggested that large texts are on the verge of extinction

because of the rate of publication of new papers, and because they can be compendious rather than critical. The latter comment applies only in part to this volume, where a few sections might have been sign-posted better, and where the literature might have been treated more sceptically. Most research workers in vision could find flaws and omissions where their speciality has been touched on, yet would surely benefit from and be stimulated by the breadth and scope of this book.

Although it is probably unfair to pick out single contributions for comment, for all the chapters are of high quality, I was particularly fascinated by the treatment given by Hughes to comparative optics. This chapter shows how much more there is to retinal organisation in mammals than the cat, and is essentially a modern critical essay in the spirit of Gordon Walls.

I applaud this book, and hope that despite its price, it will be widely appreciated by students and research workers interested in vision.

A. L. Holden

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Spectroscopic techniques

The Spectrum in Chemistry. By J. E. Crooks. Pp. 313. (Academic: London, New York and San Francisco, 1978). Hardback £12.60; paperback £6.50.

THIS is a straightforward textbook on spectroscopic techniques which are used in modern chemistry. All regions of the electromagnetic spectrum are covered, and the book is stronger than most of its rivals in the areas of X-ray spectroscopy, of photoelectron spectroscopy and of dispersion and dichroism associated with optically active materials. Indeed, chapter 9, which introduces optical activity through helices rather than asymmetrically substituted carbon atoms, is especially clear and a sufficient reason to recommend the work to undergraduate science libraries. The whole is well balanced and should be sufficient in depth for most British undergraduate courses. Thus, selection rules are briefly covered where appropriate but transition moments are not introduced. It is stronger on the general ideas and on spectroscopy in its own right than it is on quantum theory or on applications to organic or inorganic chemistry.

The author uses SI units, which is an attraction, although one wishes he did not so often remind the reader by attaching the units to the symbol for the physical quantity. For example, following equation (1.3), into which numerical values can be inserted using any set of coherent units, he adds:—"k is Boltzmann's constant in J K⁻¹". J K⁻¹ may prove convenient units but they are not essential and k stands for Boltzmann's constant in any units. The book has some 20 questions with answers. It also has its share of blemishes and errors, but these are mostly misprints or details which are wrong and do not detract from the qualitative message; they would be more important to the practitioner than an average student.

It is interesting that, as far as the reviewer can discover, Dr Crooks' personal research interests have been in rapid reactions and in solute transfer with emulsions, so that he is not an established expert in spectroscopy. There is much argument as to whether experts or others are to be preferred as undergraduate textbook writers: this book shows the demerit of the inexpert with its occasional errors, but also shows the merit of clarity and balance to be sought from 'an outsider'.

D. H. Whiffen

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Extranuclear genetics

Extranuclear Genetics. By G. Beale and J. Knowles. Pp. 142. (Edward Arnold: London, 1978.) Paperback £5.95.

FEW areas of genetics have grown so rapidly and spread so pervasively as has extranuclear genetics in the short time since it achieved some measure of respectability. Whether one dates this from the discovery of DNA in extranuclear organelles or from the publication of the first textbooks to bring the wealth of the cytogenetical evidence for extranuclear hereditary systems to a wider audience makes no difference to the time-scale. It will, however, probably colour one's reaction to the balance of material and the historical perspective which Beale and Knowles present in their account of the subject.

The bulk of the material on extranuclear genetics *sensu stricta* is contained in three chapters devoted to mitochondria, chloroplasts and miscellaneous, respectively. A further chapter on endosymbionts and viruses provides background information from extranuclear systems which may have evolutionary and certainly have phenomenological affinities with the hereditary systems located in mitochondria and plastids. A fifth chapter on plasmids resists the efforts of the authors to justify its presence. A brief introduction and conclusions complete the contents.

The authors deliberately chose to present a relatively brief resumé of recent work on *Chlamydomonas* because of the availability of up-to-date accounts. Their decision to give even briefer accounts of the extensive work on flowering plants, much of it published in German, is, however, more a reflection of the overall strategy of the presentation, with its main emphasis on the molecular and fine structure and a lesser supporting role for genetic analysis. This emphasis shows up in many ways but probably the clearest is that of the total of 36 figures, 26 of which are electron micrographs; only one is a genetic analysis and that concerns an endosymbiont. There is a corresponding shortage or delayed description of the basic details of the life cycles of key organisms without which the genetical analyses are difficult to follow. For example, it is not until more than half-way through the chapter on mitochondria and after the sections on respiratory mutants and drug resistance that the timing of meiosis in yeast and the haploidy of the ascospores are first mentioned.

But it is not this imbalance but an overcautiousness in interpretation at times bordering on scepticism that concerns me most. The high point is where, with only two possible explanations, we are told that there are almost insuperable objections to both! Personally I find this depressing, others may well applaud it as scholarly. Indeed, *Extranuclear Genetics* presents a view of the subject that many and

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perhaps all recent converts and those coming into contact with it for the first time will find palatable and a more than adequate introduction. But I doubt whether many will find it stimulating.

J. L. Jinks

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Energy concepts

The Concept of Energy. By E. J. Hoffman. Pp. 573. (Ann Arbor Science: Michigan, 1978.) £18.60.

LET it be admitted at once that this book does not exactly appeal to this reviewer. But it is clear that the author has specialised knowledge in the field. He has written on extractive distillation (1964) and on energy transformations (1970) and is known in the fuel industry. As a result, his treatment of the "mathematical origins of the concept of energy" seems to strive for a universality including philosophy and differential geometry, which the author cannot attain. The result is an uneven

book with undue elaboration of the Joule-Thomson effect (28 pages) and enthalpies of mixtures (60 pages), and nothing is said about the economic assessment of alternative energy systems, the role of energy in biological systems or the reduction in status suffered by the energy concept at the hands of relativity theory. The references are mainly pre-1962 and there are doubts, which do not represent the scientific consensus, concerning relativity (p446) and the constancy of the Stefan-Boltzmann constant (p432). Nevertheless, the considerable effort made by the author is not wasted as the book may prove to be rewarding reading for those with an interest in the specialised topics mentioned above.

P. T. Landsberg is Professor of Physics at the University of Southampton, UK.

Phase transitions in solids

Phase Transitions in Solids: An Approach to the Study of the Chemistry and Physics of Solids. By C. N. R. Rao and K. J. Rao. Pp. 330. (McGraw Hill: Maidenhead, UK, and New York, 1978.) £18.15.

ALTHOUGH the subjects treated by Rao and Rao are fascinating, I found myself somewhat disappointed by the book itself. This survey of phase transitions in solids is impressively broad, covering topics such as crystal chemistry, nucleation and spinoidal decomposition, soft modes, and the modern theory of phase transitions. Perhaps as a result, the treatment of many topics is frustratingly incomplete and sketchy. The three paragraph discussion of the Peierls transition in one-dimension systems on pp 294-5, for example, is of little value to the uninitiated. Plots of the conductivity and resistance of KCP and TTF-TCNQ are shown, without a clear explanation of what is supposed to be going on. The authors refer rather mysteriously to a "giant Kohn anomaly", with no explanation of the physics. Platitudes and vague generali-

ties apparently taken from review articles are substituted for a critical evaluation of this field.

When the book discusses subjects in more detail, it often does so uncritically. For example, "Tizsa's theory of lambda transitions" is described exhaustively, even though it is almost certainly wrong. As presented by Rao and Rao, it seems to be merely an *ad hoc* hypothesis leading to an infinite specific heat. Most lambda transitions, including the one in superfluid helium, have very singular but finite specific heats, a fact not known to Tizsa when he proposed his "theory".

Notwithstanding its faults, this book is a useful source of references and information in the fields it attempts to survey. The numerous figures and illustrations of experimental data are stimulating, although not always clearly explained. I would not recommend this book for a course on the chemistry and physics of solids, as the authors suggest in the introduction. Were it not so prohibitively expensive, *Phase Transitions in Solids* might be worth acquiring as a reference for specialists.

David R. Nelson

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J. H. TATSCH

We are pleased to announce that the first (1972) edition of *The Earth's Tectonosphere* has been completely revised. The new edition updates the results of our continuing research to determine the most probable geometrical, mechanical, thermal, and chemical aspects of the origin, evolution, and present behaviour of the Earth's upper 1000 kilometers. Included are about 80 of John C. Holden's inimitable drawings.

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Available directly from the publisher or through book dealers in all parts of the world. Hard cover, 6 x 9 inches, 468 pages. Table of contents, line drawings, maps, bibliography, index. \$30. LC: 74-78917. ISBN: 0-912890-03-7. 1977.

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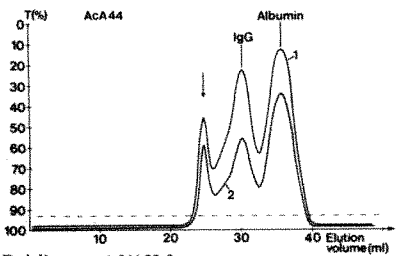
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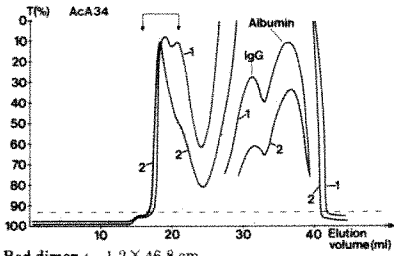
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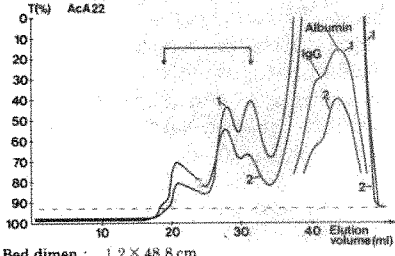
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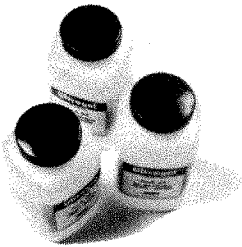
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Panasonic's AK-750 portable colour camera

Polaroid. The Polaroid CU-5 hard copy camera provides an easy, economical way for making permanent records of both repetitive and transient information from CRT screens. The CU-5 delivers a high contrast 8.3×10.8 cm photographic copy in seconds with either of two Polaroid instant black and white Land films. Type 107 C 3000 speed film that produces a finished record in 30 s that does not require coating, and Type 665—a 75 speed film that delivers simultaneously a fine grain print and a fully developed negative in 30 s without need of a dark room (the negative requires a brief clearing bath immediately after development). The computer or scope is tied up only a fraction of a second while the exposure is made. The finished print provides a permanent record as an easy-to-read print. Even dim readouts appear clear on Polaroid 3,000-speed black and white Land film. Since the prints develop outside the camera, you

can copy new information on the display immediately. This camera is available with accessory hoods for framing 8×10 cm oscilloscope screens and larger display screens up to 16.5×21.6 cm. With the addition of a power pack the CU-5 becomes a general purpose close-up camera capable of taking both colour and black and white close-up pictures from $\frac{1}{4}:1$ to three times life size. The Polaroid MP-4 camera is a copy, photomicrographic and photomicrographic recording system. It is stand mounted with eye-level reflex viewing. The new MP-4 can be converted for use as an enlarger by mounting its condenser enlarger head on the camera. Other Polaroid Land cameras and conventional cameras can be attached to the column using the universal camera mount.

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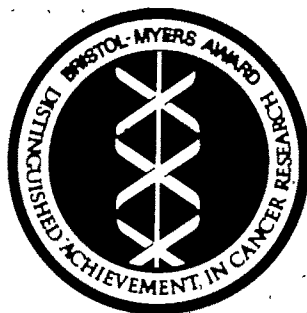
Zeiss. The Zeiss inverted microscopes IM 35 with automatic integrated 35-mm camera system and ICM 405 for fully automatic photomicrography on 35-mm or 4×5 -inch formats offer great ease of operation. Both instruments have a swing-back illuminator carrier which instantly clears the entire stage area to speed up the exchange of vessels, and allow easy micromanipulation. With condensers for long working distances, vessels and chambers of up to 20 mm thickness can be accommodated. A major advantage is the simple conversion into a high-performance fluorescence microscope. Almost any 35-mm camera back with focal-plane shutter and manual or motorised film advance can be attached.

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Gallenkamp. A new portable projection screen and a projector stand are now part of the Gallenkamp range. The screen is matt white, with a lenticulated surface which gives exceptional image definition over a wide viewing angle. The projection area is $1,220 \times 1,220$ mm and the height of the screen on its tripod stand can be adjusted between 540 and 870 mm. The screen will fold away for easy transport and storage when it is not in use. The projector stand will also fold flat. Made of strong tubular steel, it has a large table top (360×440 mm) and three adjustable height positions up to a maximum height of 1,110 mm.

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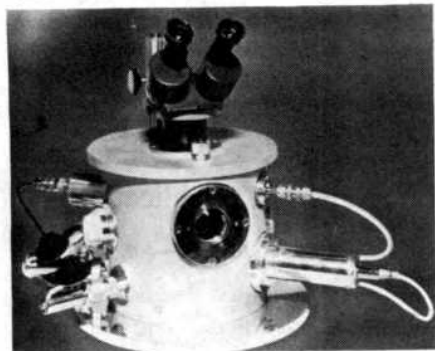


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newly on the market

Ion beam thinning unit. Ion Tech. Ion Tech announce the introduction of the Super Microlap. This incorporates two Model B11W water-cooled saddle field ion sources, controlled by a current regulated power supply unit to produce ion beams ranging in intensity from a few microamps to 500 μ A per source. For sensitive specimens, the low ion current capability and efficient water cooling guarantees freedom from ion source heat transfer and ion beam damage. The specimen can be mounted on a water-cooled or liquid nitrogen cooled stage. The ion sources operate at 10^{-4} Torr, and the fine beam is collimated by the internal electrode structure of the ion source and is independent of the cathode aperture diameter. Even after prolonged operation the ion beams remain collimated and do not spread. This equipment has the advantages of accurate ion source alignment, variable angle of incidence from normal to grazing and clean beams.

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The Super Microlap

Spectrophotometer. Perkin-Elmer. The models 554 and 555 are double beam UV/vis spectrophotometers scanning over the wavelength range 190–900 nm. The instruments feature microprocessor control of the abscissa and ordinate with keyboard entry of the operating parameters. Both instruments include automatic baseline correction, concentration calibration, auto zero, 1st and 2nd derivative, repetitive scanning and wavelength programming as standard. Abscissa and ordinate values are digitally presented and the Flowchart recorder is built-in, ensuring synchronisation of the chart with the monochromator.

Circle No. 45 on Reader Enquiry Card.

These notes are based on information provided by the manufacturers. For further details circle the appropriate numbers on the Reader Enquiry Form.

Automatic bomb calorimeter. Gallenkamp. The Gallenkamp Autobomb is an automatic instrument providing a convenient means of accurately determining calorific values to internationally accepted standards. The adiabatic system used ensures that heat losses from the calorimeter during the test period are negligible, thus eliminating the need for cooling correction calculations. Another important feature is the telescopic lift to which the top cover assembly is attached. This makes dismantling between tests unnecessary and also minimises the danger of breaking the calorimeter thermometer which need not be handled during testing. Heating is instantaneous and the water velocity through the jacket and cover is maintained at a high level to keep the temperature uniform.

Circle No. 46 on Reader Enquiry Card.

Liquid chromatography detector. Tracer. The Model 965 photo-conductivity detector for liquid chromatography, a highly selective halogen detector, has been announced by Tracer. Operating on the electrolytic conductivity principle, the detector senses any halogen-containing compound which will photolyse in the presence of UV light. The principal application will be pesticide analysis, since the detector is relatively insensitive to aromatic and other UV absorbing materials lacking halogen.

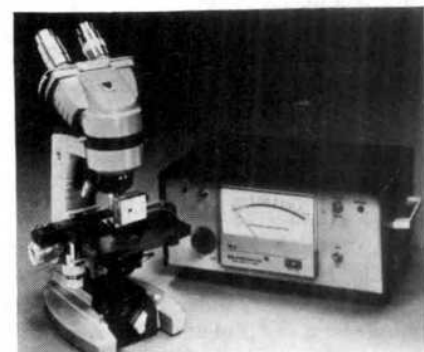
Circle No. 47 on Reader Enquiry Card.

Disposable protein concentrator. Millipore. The new immersible molecular separator II from Millipore gives the researcher a simple, rapid and economic means for concentrating or desalting solutions of proteins, macromolecule or viral suspensions. The Immersible II consists of an 11 cm² area ultrafiltration membrane cast on a porous core. The Pellicon membrane, with an exclusion limit of 10,000 daltons is specially formulated for high flux and minimum nonspecific binding. The unit is placed in the solution to be concentrated and a vacuum source connected. Low molecular weight salts and solvents pass through the membrane, while macromolecules >10,000 daltons remain in the solution. The Immersible II is available as a starter kit of 25 units with accessory tubing and connectors or as a refill kit of 25 ultrafiltration units only.

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Thermal microscope stage. Bailey. The TS-2, a solid-state thermal microscope stage cools or heats a specimen to a selected temperature within a -20°C to $+60^{\circ}\text{C}$ range and maintains that temperature indefinitely. The stage is thermoelectrically operated and produces a continuous cooling or heating effect. The TS-2 has no moving parts and is vibration free. The controller consists of a variable DC power supply and a microprobe thermometer which can be used independently for temperature measurements around the laboratory.

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The Bailey TS-2

Respiratory test meter. International Gas Apparatus. A portable meter capable of accurately measuring a patient's respiratory exhalation has been introduced by International Gas Apparatus. Based on a tried and tested principle widely used in their range of laboratory and research dry test meters, this respiratory meter is simple to operate and capable of measuring down to 0.001 cu. ft. Optional pressure and temperature gauges are available if required and various dial calibrations can be supplied to provide readings of 1/10 cu. ft or 1 cu. ft per revolution with sub-divisions of 0.001 cu. ft or 0.01 cu. ft, both models having a capacity of 115 cu. ft per hour. This test meter is designed for field or hospital use, and is only recommended for exhalatory use, not inhalation.

Circle No. 50 on Reader Enquiry Card.

Chlorophyllometer. Lars Ljungberg. The chlorophyllometer from Ljungberg is a fully transistorised photometer for the determination of chlorophylls in 100% acetone. The chlorophyllometer is a rapid and easily operated instrument with direct reading digital display (mg chlorophylls l⁻¹).

Circle No. 51 on Reader Enquiry Card.

announcements

Meetings

- 18–20 October **College Park Colloquia on Chemical Evolution**, Maryland (Dr Cyril Ponnamperna, Department of Chemistry, University of Maryland, College Park, Maryland 20742).
- 19–20 October, **9th Underwater Mining Institute**, San Diego (Gregory D. Hedden, University of Wisconsin, 1815 University Avenue, Madison, Wisconsin 53706).
- 23–26 October, **The Bourke Lectures**, Nijmegen (Faraday Division, The Chemical Society, Burlington House, London W1, UK).
- 23–26 October, **Cell Substrates and their use in the Production of Vaccines and other Biologicals**, New York (Course Secretary, W. Alton Jones Cell Science Center, Old Barn Road, Lake Placid, New York 12946).
- 23–26 October, **Biological Basis for Immunodeficiency**, Toronto (Dr E. W. Gelfand, Department of Immunology, The Hospital for Sick Children, 555 University Avenue, Toronto, Ontario, Canada M5G 1X8).
- 25–27 October, **3rd International Symposium on Polynuclear Aromatic Hydrocarbons**, Columbus (Dr Philip Leber, Battelle's Columbus Laboratories, 505 King Avenue, Columbus, Ohio 43201).
- 26–31 October, **1st International Congress on Human Ecology**, Vienna (Society for Human Ecology, Karlsplatz, A1040 Vienna, Austria).
- 27 October, **Trade Unions and Genetic Engineering**, London (ASTMS, Sutton House, 2–4 Homerton High St, London E9, UK).
- 29–October–3 November, **Vegetable Food Proteins**, Amsterdam (CEPE/AOCS, Raamweg 44, The Hague, The Netherlands).
- 29 October–3 November, **5th International Conference on Pneumonomioses**, Caracas (International Labour Office, Occupational Safety and Health Branch, CH 1211 Geneva 22, Switzerland).
- 30 October–1 November, **Physicochemical Hydrodynamics**, Indiana (Professor D. Brian Spalding, Purdue University, Thermal Sciences and Propulsion Center, Chaffee Hall, West Lafayette, Indiana 47907).
- 29 October–November, **5th International Conference on Hormonal Steroids**, New Dehli (Professor V. H. T. Jamed, Department of Chemical Pathology, St Mary's Hospital Medical School, London W2, UK).
- 31 October–4 November, **Bifurcation Theory**, New York (Ann Collins, The New York Academy of Sciences, 2 East 63rd St, New York, New York 10021).
- 1 November, **Animal Experimentation Symposium**, London (M. G. Funnell, RSPCA, The Manor House, Horsham, Sussex, UK).
- 1–2 November, **Safety and Industry**, Bath (A. Herzka, Ashbourne House, Alberon Gardens, London NW11, UK).
- 4–5 November, **Pain and Inflammation**, Bologna (Dr Emanuela Folco, Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23, 20121 Milan, Italy).
- 6–8 November, **Use of Small Accelerators**, Texas (Dr Jerome L. Duggan, Physics Department, North Texas State University, Denton, Texas 76203).
- 6–11 November, **International Workshop on Biomedical Transducers and Measurements**, Madrid (Dr Michael R. Neuman, Biomedical Electronics Resource, Engineering Design Center, Case Western Reserve University, Cleveland, Ohio 44106).
- 7–8 November, **Industrial Applications of IR Technology**, London (Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).
- 7–9 November, **Engineering Design for Earthquake Environments**, London (Keith Baldock, The Institution of Mechanical Engineers, 1 Birdcage Walk, Westminster, London SW1, UK).
- 8 November, **Food, Health and Farming**, London (Institute of Biology, 41 Queens Gate, London SW7, UK).
- 9 November, **Structure and Biochemistry of Natural Biological Systems**, Virginia (Robert L. Ryan, Philip Morris Inc., 100 Park Avenue, New York, New York 10017).
- 11–12 November, **6th Annual Down's Syndrome Congress**, Boston (Joe Canning, 35 Narragansett Avenue, Pawtucket, Rhode Island 02861).
- 13–15 November, **2nd Conference on Acoustic Emission/Microseismic Activity in Geological Structures and Materials**, Pennsylvania (Dr H. Reginald Hardy, Jr, Rock Mechanics Laboratory, The Pennsylvania State University, 104 Mineral Sciences Building, University Park, Pennsylvania 16802).
- 13–17 November, **International Symposium on the Decommissioning of Nuclear Facilities**, Vienna (IAEA, Karntner Ring 11, PO Box 590, A 1011 Vienna, Austria).

- 20–23 November, **British Crop Protection Conference — Weeds**, Brighton (Frank Bishop (Conference Planners) Ltd, 74 London Rd, Croydon, Surrey, UK).
- 20–24 November, **1st Colloquium of the European Pineal Study Group**, Amsterdam (Dr P. Pevet, The Netherlands Institute for Brain Research, Ijkdijk 28, Amsterdam–O, The Netherlands).
- 23 November, **The Future Role of Microprocessors in Nondestructive Testing**, Oxon (Clive Fewins, PR Group, Building 329, Harwell, Oxon, UK).
- 23 November, **Is Genetic Manipulation Safe?**, Bradford (Registrar, University of Bradford, Yorks, UK).
- 24–25 November, **Antibiotics and Hospitals**, Milan (Fondazione Giovanni Lorenzini, Via Monte Napoleone, 23, 20121 Milan, Italy).
- 26–29 November, **2nd International Conference on Microzonation for Safer Construction**, San Francisco (M. A. Sherrif, University of Washington, Seattle, Washington 98195).
- 27–30 November, **International Congress on Analytical Techniques in Environmental Chemistry**, Barcelona (Dr Joan Albaiges, Av. M. Cristina, Palau no. 1, Barcelona–4, Spain).

Reports & Publications

Other countries—August

- US Department of Health, Education and Welfare: National Institutes of Health. Recombinant DNA Research: Proposed Revised Guidelines. (Federal Register, Vol. 43, No. 146, July 28, 1978.) Pp 33041–33167. (Bethesda, Md.: Department of Health, Education and Welfare, Public Health Service, National Institutes of Health, 1978.) [218]
- Lincoln Laboratory, Massachusetts Institute of Technology. Seismic Discrimination—Semiannual Technical Summary Report of the Defense Advanced Research Projects Agency, 1 October 1977–31 March 1978. Pp. viii+74. (Lexington, Mass.: Lincoln Laboratory, MIT, 1978.) [218]
- World Health Organization. Technical Report Series No. 621: Epidemiology, Etiology, and Prevention of Periodontal Diseases—Report of a WHO Scientific Group. Pp. 60 Sw.fr.6; \$3. Health: A Time for Justice—Primary Health Care. Pp. 16. (Geneva: World Health Organization, 1978.) [228]
- National Multiple Sclerosis Society. Annual Report for 1977. Pp. 36. (New York: National Multiple Sclerosis Society, 205 East 42nd Street, 1978.) [228]
- United States Department of the Interior: Geological Survey. Professional Paper 1033: The Structure of the Olympic Mountains, Washington—Analysis of a Subduction Zone. By R. W. Tabor and W. M. Cady. Pp. iv+38. (Washington, DC: US Government Printing Office, 1978.) [248]
- Annals of the Transvaal Museum. Vol. 31, No. 1: The Tenebrionidae of Southern Africa, XLV—Description of Some Larvae of the Subgenera *Gonopus* and *Agonopus* of the Genus *Gonopus* (Coleoptera). By L. Schulze. Pp. 1–16+1 plate. Vol. 31, No. 2: A New Land Snail, *Curvella transvaalensis*, from Southern Africa (Gastropoda, Pulmonata: Subulinidae). By A. C. Van Bruggen. Pp. 17–20. Vol. 31 No. 3: Problematical Alcelaphine Fossils from the Kromdraai Faunal Site (Mammalia: Bovidae). By E. S. Vrba. Pp. 21–28+5 plates. Vol. 31 No. 4: A New Species of *Myosorex* from Kenya, South Africa (Mammalia: Soricidae). By J. Meester and N. J. Dippenaar. Pp. 29–42+1 plate. (Pretoria: Transvaal Museum, 1978.) [298]

Advertisement

BIOLOGICAL PACIFISM

**Complete List of Academic Works of Noted Japanese Vegetarian,
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Dr Hisatoki Komaki's academic works and activities in the field of Biological Pacifism have won him worldwide recognition, including official nomination as a candidate for the Nobel Prize, along with the respected Dr Louis Kervran of France. In 1976, Dr Komaki was cited for his role in the organisation of the Int'l Academic Congress on the Happier Coexistence of All Living Things held at Kyoto University in Japan, and at the Sorbonne, in Paris, in 1977. Prior to becoming the Founder and President of Japan's Seibutsu Noogyoo Kenkyuusho Foundation and SALT Foundation (Hisatoki Komaki Foundation for the Salvation of All Living Things), Dr Komaki, a Ph.D. from Kyoto University, was a professor at Japan's Mukogawa University.

Biological Pacifism

Dr Komaki's extensive academic research into Biological Pacifism concludes that:

1. Even in the animal kingdom, the killing of mammals (animals possessing consciousness) is exceptional.
2. A meat-diet is uneconomic from the point of view of nutrition and food production. For, animals are the consumers of all essential nutrients, and the green plants are the producers of all of them. For example, as the definition itself shows, the ESSENTIAL AMINO ACIDS—the essential components of ANIMAL PROTEIN—can never be produced by animals (Carbohydrate, too. Fat, too. Vitamins, too. Of course . . .).
3. The only role of cattle-breeding—in past days—was the biological concentration (not production!) of the nutrients of plant origin. Today, of course, we have more scientific methods for concentration of the nutrients of plant origin.
4. Famous biological scientists like Professor Dr Munro Fox, of England; Professor Dr Paul Chauchard, of France, regard the cerebral system of animals except mammals, as a kind of mechanic system like an electronic computer, possessing no consciousness. Nobody, however, can conclude that fishes, birds or insects possess no consciousness. So . . . we, mankind, must stop the slaughter of mammals right now . . . and we must stop killing all kinds of animals as soon as possible.

Biological Pacifism and the Future Religion of Mankind

In his collected works, Dr Komaki concludes that:

1. "The Future Religion of Mankind" must possess a clear vision of and a concrete method for bringing about "Peace of All Living Things."
2. The Universal Life is not only the Universal Law but also the Universal PERSONA possessing the Infinite Love, Wisdom and Power. And, therefore, the all spiritual creatures must be immortal and must evolve to be completely happy.
If not, the infinite Universe is the infinite wickedness.
3. We do not know the true mechanism. It is supposed, however, that a man is the wonderful Complex of the living brain and the Psi Body or "Psi Information System". The life span of the latter is supposed to be endless or very long, while that of the former is very short. (Prof. Dr. Hideo SEKI's English monograph on the Psi Information System Hypothesis is obtainable from him, 5-11-3, Kamiura, Setagaya, Tokyo, Japan).
4. Only those religions, possessing the firm philosophical proofs of such truths, and, furthermore, possessing the effective method of the complete manifestation, or liberation, of the True Self, will occupy the main position in (or evolve into) "The Future Religion of Mankind", providing a vision and a method of realising "Peace of All Living Things".
5. Can we find such religious or philosophical movements in the present world? Dr Masaharu TANIGUCHI's SEICHO-NO-

IYE Foundation may be one of the best academic centres.

Christianity and Buddhism also possess each vision and each method. In Christianity, via a Mediator warranted by the fulfilment of the Biblical prophecies. In Nichiren Shoshu Buddhism, via a very rhythmical and powerful chanting of "the phonetic and ideographical expression of Cosmic Life" in front of a Mandala of the Cosmic Law.

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As a result of the series of Full Page opinion advertisements on Biological Pacifism that appeared in the "New York Times" Book Review section (April 16) New Statesman (April 14), etc., etc., significant public interest has been awakened. More attention is needed, however, to achieve the goals of Biological Pacifism.

Should 500 influential citizens donate £50 or \$100 each (per one year) in support of the campaign calling for a series of the full page opinion advertisements in first class newspapers and magazines around the world, Dr Komaki's academic conclusive ideas on Biological Pacifism can be spread all over the world within a few years' time.

Please DON'T send your donation now.

Instead, if you have any constructive ideas suggesting how effectively the captioned body be organised, please send to our U.K. or U.S. Liaison Offices.



The photo-copies of Dr. Komaki's all academic works are obtainable from the central libraries of the 66 countries over the world. For the complete list of the academic works of Dr Komaki (free of charge) or for additional information write to Seibutsu Noogyoo Kenkyuusho Foundation, Sakamoto-Honmachi, Otsu, Shiga-ken, Japan or its U.K. Liaison Office, Prof. Dr G. Herriot-Hunter, The Sanctuary, Yealmpton, Plymouth, PL8 2NB South Devon, England, or its U.S. Liaison Office, c/o Mrs Ethel R. Gans, 3 Bayne St., Norwalk, Conn 06851 U.S.A.

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7157(A)

THE UNIVERSITY OF MANCHESTER RESEARCH BIOCHEMIST

Applications are invited from post-doctoral candidates for this post, to work on the mechanism of action and interaction of steroids and other hormones on bone. Work will be under the supervision of Dr D. C. Anderson, Senior Lecturer in Medicine (Endocrinology) at Hope Hospital Clinical Sciences Building, and in close collaboration with Dr S. R. Ayad, Senior Lecturer in Biochemistry. The project is for three years and will extend studies being currently conducted in this field. Previous experience with cell isolation and culture and/or hormone receptor techniques desirable. Starting salary: £3,883 to £4,382 p.a., according to age and experience, and rising according to Research Range 1A.

Send application with curriculum vitae, quoting names of two referees to Dr D. C. Anderson, Department of Medicine, Hope Hospital, Salford M6 8HD from whom further details may be obtained, by October 2, 7165(A)

INSTITUTE OF OBSTETRICS AND GYNAECOLOGY University of London Queen Charlotte's Maternity Hospital Goldhawk Road, London W6 0XG

Applications are invited for the post of
**SCIENTIFIC PROGRAMMER
Grade 1B**

at the Institute of Obstetrics and Gynaecology, Queen Charlotte's Maternity Hospital.

Salary scale £3,384 to £5,604 plus £450 London Weighting Allowance.

The primary function of this post is to support a new PDP11 programmable remote-job-entry terminal connected to the University CDC computer installation. The work will involve a wide variety of software development using assembler and high level languages, plus experience of real-time programming. The successful applicant will work in a small, lively research department with a lot of scope for inventive development.

A scientific background (to Honours degree level) would be an advantage. Registration of a suitable candidate for a higher degree would also be a possibility.

Further details may be obtained from the Secretary, Institute of Obstetrics and Gynaecology, Chelsea Hospital for Women, Dovehouse Street, London SW3 6LT.

Applications, together with curriculum vitae and the names and addresses of 2 referees should be sent to: Dr G. S. King, Chemical Pathology Department, Queen Charlotte's Maternity Hospital, Goldhawk Road, London W6 0XG, Monday, October 16, 1978. 7213(A)

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Duties will involve the forwarding of the subject, the teaching of medical students, conducting research, and the administration of the Department.

It is intended that the person appointed will take up his or her duties as soon as convenient, and be offered an honorary consultant contract with the Ealing, Hammersmith and Hounslow Area Health Authority (Teaching).

Further details should be obtained from the Academic Registrar (N Senate House, Malet Street, London WC1E 7HU before submitting applications (10 copies). Closing date November 20, 1978. 7163(A)

UNIVERSITY OF SOUTHAMPTON

DEPARTMENT OF CHEMISTRY

Postdoctoral Research Fellow to work with Drs M. C. Flowers and J. S. Ogden on a project supported by the Science Research Council and concerned with the investigation of the chemistry of laser evaporated species. The work involves the use of quadrupole and time of flight mass spectrometers and experience in mass spectrometry would be an advantage. The appointment is for one year commencing not later than December 1978. The salary will be £3,883 p.a. Applications with full curriculum vitae and the names of two referees should be sent either to Dr M. C. Flowers or Dr J. S. Ogden, Department of Chemistry, The University, Southampton SO9 5NH, as soon as possible. Please quote ref MA1/2/Na. 7174(A)

ADDRESS

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20 on Reader Enquiry Card



National Research Council Canada
Conseil national de recherches Canada

1979 RESEARCH ASSOCIATESHIPS

for research in science and engineering in the following laboratories of the NATIONAL RESEARCH COUNCIL OF CANADA:

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DIVISION OF BIOLOGICAL SCIENCES
DIVISION OF BUILDING RESEARCH
DIVISION OF CHEMISTRY
DIVISION OF ELECTRICAL ENGINEERING
HERZBERG INSTITUTE OF ASTROPHYSICS
DIVISION OF MECHANICAL ENGINEERING
NATIONAL AERONAUTICAL ESTABLISHMENT
DIVISION OF PHYSICS
PRAIRIE REGIONAL LABORATORY

Applicants should possess at least a Ph.D. in natural science or a Master degree in an engineering field or expect to obtain the degree before taking up the award.

Associateships are open to nationals of all countries although preference will be given to Canadians.

Research Associates will be offered appointments to the staff of the National Research Council on a term basis and will be offered salaries and benefits currently available to members of the continuing staff.

The initial appointment will be for a one year term and may be renewed, subject to the Associate's performance and subject to the requirements of the Division. Renewals are considered annually. The term as a Research Associate will vary by Division but will not exceed five years.

An allowance will be provided towards the cost of travel.

Applications may be obtained from the Research Associates Office, National Research Council of Canada, Ottawa, Canada, K1A 0R6.

Closing date: 15 January 1979

7155(A)

REGIONAL CYTOGENETICS LABORATORY EAST BIRMINGHAM HOSPITAL LOCUM SCIENTIFIC OFFICER (Basic Grade)

Applications are invited for the locum post of Scientific Officer, initially for 3 months, with the possibility of a permanent post after 6 months. Experience in cytogenetics is essential but not absolutely necessary. Salary range depending on experience and qualifications, £2,991 to £4,899 p.a.

Application forms and further details available from the Regional Cytogenetics Laboratory, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST. Tel 021-772 4311 ext. 4354. 7192(A)

UNIVERSITY OF ABERDEEN DEPARTMENT OF BIO-MEDICAL AND BIO-ENGINEERING RESEARCH ASSISTANT

Applications are invited for this post which is supported by the Cancer Research Campaign for the investigation of the properties of overlapping ultrasonic fields and the instrumentation for producing and measuring them. Candidates should have first degree in Physics and Ph.D. in Physics or Bio-Physics or Electronics. The post is tenable initially for two years.

Commencing salary up to £4,300 per annum on the Range 1A Scale for Research and Analogous Staff, plus U.S.S.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by October 13, 1978. 7146(A)

LOTHIAN HEALTH BOARD METABOLIC UNIT

Western General Hospital

Applications are invited from candidates with an Honours degree in chemistry, biochemistry, or a biological science for the appointment of

BIOCHEMIST

(Basic grade)

In the above unit which is concerned with the management of and research into endocrine and metabolic disorders. Suitable candidates may register for a higher degree and may take part in the M.C.B. training programme run by the Departments of Clinical Chemistry.

Whitley Council conditions of service apply.

Further particulars may be obtained, or a visit arranged by contacting Professor J. A. Strong, University Department of Medicine, Western General Hospital, Crewe Road, Edinburgh EH4 2XU. Telephone: 031 332 2525, ext. 365.

Applications, which should be typewritten, giving the names and addresses of two referees should be lodged with the Secretary, 11 Drumshough Gardens, Edinburgh EH3 7QQ, by October 5, 1978. 7208(A)

UNIVERSITY OF LANCASTER DEPARTMENT OF ENVIRONMENTAL SCIENCES RESEARCH ASSOCIATE

A physicist at Ph.D. level is required for a programme of studies of the high-altitude ionosphere supported by the Science Research Council.

Applicants should be capable of managing a small observational programme, including data handling, as well as carrying through scientific ideas based on observational data. A research programme is essential.

The appointment will commence as soon as possible and terminate on February 28, 1982. Initial salary £130 (under review).

Further particulars may be obtained by writing reference L79/D) from the Establishment Officer, University House, Lancaster LA1 4YW, to whom applications (five copies), naming three referees, should be sent not later than October 9, 1978. 7167(A)

CSIRO AUSTRALIA Research Scientist

Division of Animal Health Armidale, NSW

CSIRO has a broad charter for research into primary and secondary industry areas. The organisation has approximately 7,000 employees—2,400 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Immunology/Microbiology

General: The Division is currently reorganising its research programmes at McMaster Laboratory, Sydney and the Pastoral Research Laboratory, Armidale. The group at present comprises four senior parasitologists and an immunologist and it has been decided to diversify the activities of the group by the appointment of a second immunologist/microbiologist to work on aspects of staphylococcal disease. It is expected that the new appointee will develop some association with colleagues at the McMaster Laboratory in Sydney.

Duties: To carry out research into various aspects of staphylococcal immunity with particular reference to staphylococcal mastitis. Attention will be directed to determining the relative importance of various staphylococcal antigens as immunogens and to improving vaccination procedures.

Qualifications: A Ph.D. degree in a relevant discipline, or equivalent qualifications, supported by satisfactory evidence of research ability and experience appropriate to the field of study.

Salary: Research Scientist or Senior Research Scientist: \$A14,829 to \$A21,643 p.a. An outstanding applicant may be considered for appointment at a higher level.

Tenure: Indefinite with superannuation.

Applications **IN DUPLICATE**, stating **FULL** personal and professional details, the names and addresses of at least two professional referees, and **QUOTING REFERENCE NUMBER 201/563** should reach: The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON, WC2R 3EH, by October 23, 1978.

Applications in U.S.A. and Canada should be sent to: The Counsellor (Scientific), Embassy of Australia, 1801 Massachusetts Avenue, N.W., WASHINGTON, D.C., 20036, U.S.A. 7146(A)

HARPER ADAMS AGRICULTURAL COLLEGE Newport, Salop

LECTURER GRADE 2 in CROP PROTECTION

Applications are invited for a new post as Lecturer in Crop Protection. The person appointed will have responsibility for laboratory and field instruction and experimentation.

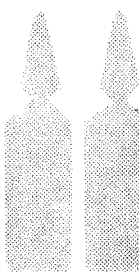
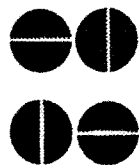
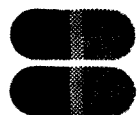
Candidates should have a degree in Agriculture or Horticulture together with postgraduate training and experience in Crop Protection.

Salary in Burnham F.E. Scale £4,101 to £6,658, point of entry dependent on qualifications and experience.

Further details and application form from The Bursar, Harper Adams Agricultural College, Newport, Salop, TF10 8NB. 7207(A)

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Librarian

An opportunity has arisen for a Chartered Librarian to take charge of our Company Library at Ware.

The Library contains mainly scientific books and journals. It provides a service to scientists qualified in Biology, Chemistry, Medicine and Pharmacy, and also to members of Glaxo Operations Limited, a production Company on the same site. We are shortly to move into a modern, custom-built library which will have microfilm and reprographic equipment.

Candidates must be Chartered Librarians and have several years' relevant special library experience.

The position carries a competitive salary which will reflect the age and experience of the successful candidate. Other benefits include Flexitime working, pension and profit sharing schemes, extensive sports and social facilities, and assistance with relocation expenses where applicable.

Please apply to:
Dr Ian Collins, Personnel Manager,
Glaxo-Allenburys Research (Ware) Ltd.,
Ware, Herts. SG12 0DJ.
Telephone Ware 3232.

Glaxo-Allenburys Research (Ware) Limited

7178(A)

UNIVERSITY COLLEGE CARDIFF

Applications are invited for the post of
**POSTDOCTORAL
RESEARCH ASSISTANT**

to study collagen gene expression in cultured human chondrocytes in the DEPARTMENT OF BIOCHEMISTRY. The appointment, which is funded by a grant from the M.R.C. to Dr R. Harwood, is tenable for 3 years and is to be made as soon as possible. Previous experience of connective tissue or nucleic acid biochemistry would be advantageous, though not essential.

Applications (2 copies), together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further particulars may be obtained. Closing date October 12, 1978. Please quote reference 1598. 7171(A)

GERMAN CANCER RESEARCH CENTER HEIDELBERG. POSTDOCTORAL RESEARCH ASSISTANT

Dept.: Molecular Biology
of DNA-Tumor-Viruses
Dr Gerhard Sauer, Head

Applications are invited from biologists or biochemists for the above post. The work will involve studies on the integration of the viral genome in the host cell DNA. The salary will be within the range of DM 2,900 to DM 3,100 depending on age. The appointment will be for up to two years starting on October 1, 1978.

Applications, stating age, academic qualifications and the names of two referees should be sent to Department of Administration, Deutsches Krebsforschungszentrum, 6900 Heidelberg, Im Neuenheimer Feld 280, Germany. 7201(A)

ST MARY'S HOSPITAL MEDICAL SCHOOL (University of London) Paddington W2 1PG DEPARTMENT OF PHYSIOLOGY POSTGRADUATE RESEARCH ASSISTANT

Applications are invited for the above post from candidates with a good Honours Degree in an appropriate field. The post is funded for a period of two years only by The Wellcome Trust and is available from October, 1978. The work involves research on the Physiology of Foetal Development. Salary would commence at £3,214 p.a. plus £450 p.a. London allowance. Applications, including a full curriculum vitae with the names and addresses of two referees to the Secretary, St Mary's Hospital Medical School, Paddington, London W2 1PG, quoting ref. N/HB/J. 7176(A)

UNIVERSITY OF NOTTINGHAM DEPARTMENT OF APPLIED BIOCHEMISTRY AND NUTRITION FOOD SCIENCE LABORATORIES A.R.C. RESEARCH ASSISTANTSHIP

tenable from October, 1978.

Applications are invited for the above post to study the dynamics of starch gelatinisation using one or more novel physicochemical techniques.

Candidates may register for a Ph.D. degree but should preferably have a good honours degree in Chemistry, Physics or some related subject.

Salary £3,384 to £3,883.

Applications, naming two referees, should be sent as soon as possible to: J. M. V. Blanshard, Dept. of Applied Biochemistry and Nutrition, University of Nottingham, School of Agriculture, Sutton Bonington, Loughborough, Leics. LE12 5RD, from whom further details may be obtained. 7175(A)

THE QUEEN'S COLLEGE GLASGOW LECTURER

with good honours degree in Nutrition or Dietetics or a related science and preferably eligible for State Registration in Dietetics to teach Applied Nutrition and Diet Therapy at Degree and Diploma level. This is a new appointment.

Salary range: £4,056 to £7,167 (bar) to £7,698 per annum. (Full credit will be given for all relevant experience when calculating placement on this scale.)

A senior appointment may be available for an exceptional candidate. Assistance with removal expenses.

Further details and forms of application may be obtained from The Principal, 1 Park Drive, Glasgow G3 6LP to whom completed forms should be returned not later than Monday, October 16, 1978. 7149(A)

THE UNIVERSITY OF MANCHESTER DEPARTMENT OF PSYCHIATRY RESEARCH FELLOW IN NEUROPHYSIOLOGY

Applications are invited for this post (tenable until December 1980) to study the electrophysiological consequences of adrenoceptor activation in mammalian nerve cells using intracellular recording techniques. The appointee will collaborate with a small research team working within the field of cellular neuropharmacology. Applicants should possess a Ph.D., and have had research experience with neurophysiological techniques. Preferred starting date: January 1.

Salary range p.a. £3,883 to £6,555. Applications, with curriculum vitae and the names of two referees should be sent before October 31, to Dr E. Szabadi, Department of Psychiatry, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT. 7166(A)

MAX-PLANCK-INSTITUTE West Berlin

There is a vacancy for a
**PHYSICAL CHEMIST
or BIOCHEMIST**

to work on physical properties of proteins and nucleic acids. Applicants with previous experience in this field will be preferred.

The position is available from January 1979 and is initially for 5 years with the possibility of renewal. Salary will be in the range 44,000DM/year (=£11,000=US\$22,000).

Applications are invited before December 1978 giving information about qualifications and the address of 2-3 referees. They should be sent to: Max-Planck-Institut für Molekulare Genetik, Personalbüro, Innestraße 63-73 D-1000 Berlin 33, Germany. 7209(A)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF PATHOLOGY DIVISION OF VIROLOGY RESEARCH TECHNICIAN

required from October 1, or as soon as possible, thereafter to join an active group studying the regulation of influenza virus gene expression. Qualifications: good degree, HNC or equivalent in biochemistry or microbiology. Experience in tissue culture or virology advantageous.

Starting salary up to £2,959 p.a. annum.

Applications and enquiries to B. W. J. Mahy, Division of Virology, New Addenbrookes Hospital, H. Road, Cambridge CB2 2QQ. Telephone 0223-45171, extension 313. 7211(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF MICROBIOLOGY

Applications are invited for the post of a postdoctoral Research Fellow in the Department of Microbiology.

The research which is under the direction of Professor H. R. Perkins will be concerned with the structure and biosynthesis of the bacterial cell envelope in relation to antibiotic action.

The post is financed by the M.R.C. and the initial salary will be within the range £3,883 to £5,129 per annum.

Applications, together with the names of two referees, should be received not later than October 9. The Registrar, The University, P. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/929/N. 7159(A)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF BIOCHEMISTRY RESEARCH ASSOCIATE

Applications are invited for the post of RESEARCH ASSOCIATE for work on the role of the recA and leu genes of E. coli in DNA repair and genetic recombination. The project is supported by a grant from the M.R.C. and the position is tenable for three years at a salary on the scale £3,883 to £6,555 under review. The work will involve combination of genetic and biochemical techniques and previous experience in protein purification, molecular cloning and DNA sequencing would be particularly advantageous.

Applications, including a curriculum vitae and the names of two referees should be sent to Dr P. T. Emmers, Department of Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU. 7160(A)

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**UNIVERSITY OF
NEWCASTLE UPON TYNE
DEPARTMENT OF
AGRICULTURAL BIOLOGY
RESEARCH ASSOCIATE**

Applications are invited for a postdoctoral Research Associate in the Department to study the effects of climatic factors on the growth of clover, in a project supported by the Agricultural Research Council. Experience in micrometeorological techniques would be an advantage. The appointment will be for 3 years commencing October 1, or as soon as possible thereafter, at a salary within the Range 1A scale £3,883 to £6,555 (under review). Further particulars may be obtained from the Registrar, University of Newcastle upon Tyne NE1 7RU, to whom applications, together with curriculum vitae (three copies) and the names of three referees, should be sent as soon as possible. Please quote reference AGRIC/160 on all communications. 7212(A)

**UNIVERSITY OF BATH
SCHOOL OF CHEMICAL
ENGINEERING
TECHNICIAN**

A vacancy has arisen on a two year contract to assist in the development and design of a protein recovery process. Candidates should be qualified in Chemistry or Chemical Engineering to at least O.N.C., or 'A' level standard with industrial, or similar experience. Starting salary in the range £2,680 to £2,835 per annum (under review) depending on qualifications and experience. Application forms are obtainable from the Personnel Officer, University of Bath, Bath BA2 7AY, quoting reference number 78/184R/N. Closing date will be October 9, 1978. 7169(A)

**HARVARD SUMMER
SCHOOL
POSITIONS IN BIOLOGY**
Applications are invited for Summer school teaching positions in Biology. Introductory courses are emphasised with possibility of intermediate offerings. Possible courses include: general biology, zoology, botany, cell biology, physiology, and genetics. Compensation at Harvard median. Applications including C.V. should be sent to: Dr Marshall R. Pihl, Associate Director, Harvard Summer School, 20 Garden Street, Cambridge, Mass. 02138. DEADLINE: October 31, 1978. Harvard University is an Equal Opportunity/Affirmative Action Employer. 7170(A)

**UNIVERSITY OF LEICESTER
THE MEDICAL SCHOOL
DEPARTMENT OF ANATOMY**
A vacancy exists for a POSTDOCTORAL RESEARCH ASSOCIATE to work with Professor Felix Beck on embryonic nutrition and teratology. The project, financed by the R.C., will involve embryonic culture, autoradiography and electron microscopy. Candidates should have experience of A.R.G. in particular. Initial salary £3,660 a year (under review). Applications to Professor F. Beck, Department of Anatomy, University of Leicester LE1 7RH, as soon as possible. 7205(A)

**THE UNIVERSITY OF
SHEFFIELD
DEPARTMENT OF
BUILDING SCIENCE
Building Aerodynamics
Research**

Applications are invited from men and women for a Research Post in the field of Building Aerodynamics. This post has been created by a Building Research Establishment contract to investigate the dynamic wind loads on buildings using wind tunnel techniques and is currently tenable, for a three year period. Some postgraduate experience in the use of experimental methods and data analysis is desirable and an appropriate background would be expected in one of the engineering or applied physics disciplines. Salary, dependent on age and qualifications, for a Postdoctoral appointment: on Range 1A up to £4,130 p.a.; for a Postgraduate appointment on Range 1B up to £3,660 p.a. These salaries will have anomalies correction of approximately 6% added to them following October 1, 1978. Further particulars may be obtained from the Registrar and Secretary, University of Sheffield, Sheffield S10 2TN to whom applications should be sent by October 6, 1978. Quote ref: R 199/G. 7191(A)

**UNIVERSITY OF
LIVERPOOL
DEPARTMENT OF
METALLURGY AND
MATERIALS SCIENCE**
Applications are invited for two posts of Senior Research Assistant. (a) On a project concerned with the effects of gas-ion bombardment on the microstructure of materials. Experience of transmission electron microscopy at the postgraduate level will be required. (b) To undertake theoretical research on an S.R.C. sponsored project concerned with the structure of defects in crystalline polymers. The initial salaries will be within the range £3,883 to £4,882 per annum. Applications should be received as soon as possible, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote. Ref: RV/932, A or B/N. 7193(A)

**UNIVERSITY OF BRISTOL
TSETSE RESEARCH
LABORATORY, LANGFORD
INSECT PHYSIOLOGISTS**
Applications are invited for two research assistantships to work with Dr P. A. Langley on various aspects of the physiology of tsetse flies. Areas of research are exploitation of the sex recognition pheromone in development of new control measures and the role of the neuroendocrine system in control of reproduction in tsetse. The first post is tenable from January 1, 1979 and the second at a date to be arranged in 1979, for three years. Selected candidates will be graduates with a first or upper second class honours degree and will have the opportunity to register for a higher degree within the University of Bristol. Salary will be commensurate with age and experience on the appropriate scales for research and analogous staff, e.g. £3,384 per annum at age 24. Applications, with curriculum vitae and the names of two referees, should be sent, before October 31, 1978 to The Director, Tsetse Research Laboratory, Langford House, Langford, Bristol BS18 7DU. 7183(A)

Lead a small, elite taskforce in high-temperature materials chemistry research.

If you're (1) a recognized leader in your own area for research, (2) have a strong background in physical and solid state chemistry including modern thermo-dynamics, and (3) have successfully lead group research efforts... you may well be the candidate we're seeking.

We're the Chemistry Division of Argonne National Laboratory—one of the nation's largest multi-disciplinary centres for basic and energy-related research. As the successful candidate for our High-Temperature Materials Chemistry group you'll be assuming Staff/Group Leadership responsibilities within a year of your appointment. You will also have an opportunity to develop new basic research programs and carry out your own research work.

Immediate specific responsibilities include leading a group of six highly-qualified researchers, and co-ordinating experimental and theoretical studies of:

- (A) Lattice defect and electronic structures of non-stoichiometric phases.
- (B) Thermodynamic properties of inorganic solids, liquids and gases at temperatures from 500 to 2,500°C.
- (C) Transport properties (diffusion and conductivity) of non-stoichiometric phases and kinetics of solid-gas reactions.
- (D) Interpretive and computational thermodynamic studies on the derivation of equations of state and transport.

For prompt, confidential consideration of your qualifications please send detailed resume outlining academic and other credentials to: Dr. Arthur D. Tevebaugh, Associate Director, Chemistry Division or to Mr.

Ronald A. Johns, Manager Employment Placement, Argonne National Laboratory, 9700 S. Cass Avenue, Argonne, IL 60439.

An equal opportunity employer, m/f 7204(A)



**ARGONNE
NATIONAL
LABORATORY**

**GREATER GLASGOW HEALTH BOARD
EASTERN DISTRICT
RESEARCH BIOCHEMIST**
Glasgow Royal Infirmary, Glasgow G4 0SF
DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the above post to develop tests of thyroid function suitable for a programme of neo-natal screening for congenital hypothyroidism in Scotland. This collaborative project involving the department of Pathology, Biochemistry and Medical Genetics, University of Glasgow and Department of Bacteriology, Stobhill General Hospital, Glasgow is supported by the Biomedical Research Committee of the Scottish Home and Health Department.

Applicants should have a good honours degree in Biochemistry or related subjects and preferably a higher research degree. The salary will be within the basic grade scientist scale commencing £3,486 to £4,275.

Candidates are invited to visit the laboratories by arrangement with Dr J. G. Ratcliffe, Telephone No. 041-552 3535, Extension 5275, from whom further particulars are available.

Applications in writing giving two names for reference should be submitted to Dr J. G. Ratcliffe, Department of Biochemistry, Glasgow Royal Infirmary, Glasgow G4 0SF within two weeks of appearance of this advertisement. 7147(A)

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Applicants, male or female, must have instrumentation experience in one of the above-mentioned areas, and be aged 23-35 years with a recognised qualification.

The successful candidates will provide a technical and practical sales support to our Field Sales Force by running instrument demonstrations at our Beaconsfield facilities and at customer's laboratories when necessary. They will also be called upon to run product seminars and staff exhibitions.

I would be pleased to assist you in matching your qualifications and experience to our most suitable vacancy—so, if our products interest you and you would like to join a team of lively and energetic people, why not write to me or telephone for an application form.

7156(A)

Please apply to: Allan Brooks, Snr. Personnel Officer, Perkin-Elmer Limited,
Post Office Lane, Beaconsfield, Bucks. Tel: Beaconsfield 6161

COMMONWEALTH AGRICULTURAL BUREAUX Vacancy for SCIENTIFIC INFORMATION OFFICER at the

Commonwealth
Institute of Helminthology
The White House
103 St. Peter's Street,
St. Albans, Herts.

Duties: To assist in the preparation of *Helminthological Abstracts*, Series 1 Plant Nematology, and the Institute other publication in nematology.

Qualifications: Good degree in biology and ability to write clear and concise English essential. Training in plant nematology and knowledge of one or more other European languages advantageous.

Salary: In scale £2,839 to £5,448 plus London Weighting of £275 per annum and a compensatory allowance (taxable but not superannuable) of 3.3% of basic salary to offset personal contribution to Superannuation Scheme. Starting salary according to age, experience and qualifications.

Application forms and details from:
The Director,
Commonwealth Institute
of Helminthology,
The White House,
103 St. Peter's Street,
St. Albans,
Herts, AL1 3EW.
Closing date, October 20, 1978.
7210(A)

CHELSEA COLLEGE University of London POSTDOCTORAL RESEARCH ASSISTANT

is required to join a group working on lipids of plant membranes. Particular experience in the biochemistry and biophysics of lipids and lipid-water systems would be an advantage. The post is supported by the Agricultural Research Council and the appointment will be for a project approved for a period of three years. Salary in the range £3,660 to £4,130 per annum plus £450 London Allowance. Application forms and further details from the Personnel Officer, Chelsea College, Annexe, Friese Green House, Chelsea Manor Street, London SW3 3TW. Closing date October 9, 1978.

7164(A)

Applications are invited for: FREE-LANCE EDITORIAL WORK

on scientific manuscripts and proof for the Geology Department. Essential requirements are English as mother tongue, an appreciation of good English style, a meticulous eye for detail, and some background in Earth Sciences. Applications, in writing, with details of experience and qualification (including specialist field of study should be addressed to Mr P. Hardorff Elsevier Scientific Publishing Company, P.O. Box 330, 1000 AA AMSTERDAM, The Netherlands.
7168(A)

RESEARCH TECHNICIAN for Department of Anatomy to assist group in study of the development of lymphocytes. Extensive tissue culture and the use of a variety of immunological techniques are involved. Previous experience and appropriate qualifications essential.

Instruction will be given in techniques related to Tissue Culture and Immunology. Salary in the range of £2,689 to £3,087.

Ref. 401/C/327. Application form from, Assistant Secretary, Personnel Office, University of Birmingham P.O. Box 363, Birmingham.

7188(A)

EEC PHARMACEUTICAL COMPANY

seeks

DIRECTOR OF RESEARCH

The successful candidate will report directly to the Managing Director and should have particular capabilities in:

- Creativity and initiative substantial experience in pharmaceutical research.
- Strong background in pharmacology.
- The company is medium sized, located in Paris and is already actively engaged in international development and licensing.
- It's research facilities employ approximately 100 people.
- Nationality indifferent, fluent English and French essential.

Please write to: SODECI, 140 Rue de la Croix Nivert, 75015 Paris (France).

7214(A)

HARPER ADAMS AGRICULTURAL COLLEGE Newport, Salop

SENIOR LECTURER in AGRICULTURAL CHEMISTRY

Applications are invited for the above post available from January 1, 1979. The person appointed will be responsible for the teaching and research work in the Agricultural Chemistry Unit.

Candidates should have a degree in Agricultural Chemistry with a particular interest in Animal Nutrition.

Salary in Burnham P.E. Scale £6,051 to £7,315, point of entry dependent on qualifications and experience.

Further details and application form from: **The Bursar, Harper Adams Agricultural College, Newport, Salop, TF10 8NB.**

7206(A)

KING'S COLLEGE HOSPITAL MEDICAL SCHOOL (University of London) Denmark Hill, London SE5 8RX RESEARCH BIOCHEMICAL TECHNICIAN

to work in Cardiac Research Laboratory in King's College Hospital (Dr D. Jewitt). Project involves assessment of new drugs designed to prevent abnormal heart rhythms after coronary thrombosis. The technician will be responsible for plasma drug level estimations. Experience of spectrofluorimetric and gas chromatographic techniques an advantage. H.N.C. qualification required. Salary according to age, qualifications and experience. Further details may be obtained from Dr D. Jewitt, telephone 01-274 6222 ext. 2500.

Applications to the Secretary of the Medical School at the above address. Please quote reference EJ/N.

7162(A)

UNIVERSITY OF BIRMINGHAM FACULTY OF SCIENCE AND ENGINEERING CHAIR AND HEADSHIP OF THE DEPARTMENT OF MATHEMATICAL STATISTICS

Applications are invited for the Chair and Headship of the Department of Mathematical Statistics which will become vacant from October 1, 1978 on the retirement of Professor H. E. Daniels.

Salary in the professorial range plus superannuation.

Further particulars available from the Registrar, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (12 copies: 1 from overseas applicants), naming three referees, should be sent by October 30, 1978.

7143(A)

CHANGE OF ADDRESS

The Advertisement offices for NATURE have moved to
3 DYERS BUILDINGS, LONDON, EC1N 2NR
TELEPHONE : 01 831-6901

POSTDOCTORAL POSITION

Available immediately for research isolation and characterisation of lymphocyte membrane receptors. A strong background in immunology or protein chemistry is required. Send résumé with three references to:

Dr J. J. Marchalonis
Position 1035
Cancer Biology Program
FREDERICK CANCER
RESEARCH CENTER
PO Box B
Frederick, Maryland 21701
Equal Opportunity Employer M/F
7189(A)

UNIVERSITY COLLEGE LONDON DEPARTMENT OF PHYSICS AND ASTRONOMY TWO POSTDOCTORAL RESEARCH ASSISTANTS

Required as soon as possible for the development of systems and applications software for the Interactive Analytical Image Processing System (IAPS), being built jointly by the Appleton Laboratory and University College London. Candidates should possess a Ph.D.; also for applications software preferably a good knowledge of dynamical meteorology, image processing and FORTRAN programming and for systems software a good knowledge of systems programming. Starting salaries will be in the range £3,359 to £4,858 including London Allowance.

Applications with Curriculum Vitae and the names and addresses of two referees should be sent to Dr G. E. Hunt, Department of Physics and Astronomy, University College London, Gower St., London WC1E 6BT.
7197(A)

UNIVERSITY OF SURREY DEPARTMENT OF BIOCHEMISTRY RESEARCH FELLOW

Applications are invited for a position to work with Dr R. S. Jones and Professor D. V. W. Parke on an R.C. supported project concerned with an aspect of environmental carcinogenesis. Applicants (male or female) should be graduates of either chemistry or biochemistry and possess a Ph.D./D.Phil. Experience in P.L.C. and/or other analytical techniques would be beneficial but not essential.

The appointment is for a period of 3 years, commencing on January 8, 1979 at a starting salary of £3,660 per annum (Range 1A).

Applications, in the form of a curriculum vitae, including the names and addresses of two referees, should be sent to the Assistant Secretary (Personnel), University of Surrey, Guildford, Surrey GU2 5XH to be received by: October 5, 1978.

Further information may be obtained from Dr R. S. Jones, in the Department of Biochemistry.
7181(A)

UNIVERSITY OF ABERDEEN RESEARCH ASSISTANT IN PHYSIOLOGY

Applications are invited for the above post from Graduates in Physiology, Pharmacology or Biochemistry, to work with Dr D. M. Campbell and Dr M. A. Radcliffe on maternal fat metabolism in pregnancy. Appointment is tenable for three years to commence as soon as possible.

Commencing salary £3,384 rising to £3,883 per annum, on Range 1B Scale of Research and Analogous Staff.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by October 6, 1978.
7144(A)

UNIVERSITY COLLEGE CARDIFF DEPARTMENT OF PLANT SCIENCE POSTGRADUATE OR POSTDOCTORAL BIOCHEMIST

Applications are invited for a POSTGRADUATE or POSTDOCTORAL

RESEARCH ASSISTANTSHIP to work on nucleic acid metabolism in tomato seeds under the supervision of Dr J. A. Bryant. The post is available for 15 months in the first instance, with the possibility of renewal. Salary within Research and Analogous Staff Range 1B: £3,384 to £4,631. Duties to commence as soon as possible.

Applications (2 copies), together with the names and addresses of two referees, should be forwarded to the Vice-Principal (Administration) and Registrar, University College, P.O. Box 78, Cardiff CF1 1XL, from whom further particulars may be obtained. Closing date October 5, 1978. Please quote reference 1601.
7184(A)

ROYAL POSTGRADUATE MEDICAL SCHOOL (University of London)

LECTURER IN ANALYTICAL CHEMISTRY IN THE DEPARTMENT OF CLINICAL PHARMACOLOGY

Applications are invited for the above post, from persons possessing a Ph.D. degree in Chemistry or Biochemistry, and postdoctoral research experience in the use of sophisticated analytical techniques, especially GC-MS, in medical research. Some knowledge of synthetic organic chemistry would be an advantage. The successful candidate will be responsible for the direction of the Department's analytical laboratory which is equipped with a full range of instruments including Finnigan 3200 and Finnigan 4000 GC-MS-computer systems, and will also be encouraged to develop an independent line of research. Salary in the range for non-clinical lecturers (from October 1, 1978): £3,883 to £7,754 a year plus £450 London Allowance. Superannuation under U.S.S.

Applicants should apply by letter enclosing six copies of their curriculum vitae, to the Secretary, Royal Postgraduate Medical School, Hammersmith Hospital, Du Cane Road, London W12 0HS, quoting ref. no. 20/3/N. Closing date October 7, 1978.
7152(A)

EAST MALLING RESEARCH STATION PLANT PATHOLOGY DEPARTMENT A VIROLOGIST

to join team working on virus and virus-like diseases of fruit and hop plants.

Applications invited from graduates with honours degree or ordinary degree or HNC in virology, microbiology, or plant pathology.

Appointment Scientific Officer grade (£2,839 to £4,415) or Higher Scientific Officer grade (£4,101 to £5,448) depending on qualifications and experience.

Details and application form from Secretary, East Malling Research Station, East Malling, Maidstone, Kent ME19 6BJ.
7176(A)

Scientific Officer/ Higher Scientific Officer £2,839 to £5,448

Applications are invited for a pensionable post in the Field Botany Division of the Department of Agriculture situated at Newforge Lane, Belfast.

The successful candidate will carry out research in the field and under controlled environmental conditions aimed at increasing productivity and utilisation of pasture in hill land in Northern Ireland. The successful candidate may also be required to undertake lecturing duties in the Faculty of Agriculture and Food Science, Queen's University, Belfast.

HIGHER SCIENTIFIC OFFICER

Under 30 years of age at 31 December 1978 and possess a 1st or 2nd Class Honours Degree in Agricultural Botany, Agriculture or Botany and at least 2 years' appropriate postgraduate experience.

SCIENTIFIC OFFICER

Under 27 years of age at 31 December 1978 with an Honours Degree as above.

Preference may be given to candidates with knowledge of soil science.

Exceptionally applications may be considered from candidates over the age limits who have specialised experience.

SALARY SCALES

Higher Scientific Officer—£4,101 to £5,448

Scientific Officer—£2,839 to £4,415

Please write or telephone for an application form quoting job reference SB 314/78/N to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR. (Telephone Dundonald 4585 ext. 256). Completed forms must be returned to arrive not later than October 5, 1978.
7142(A)



Scottish Institute of Agricultural Engineering SOILS SECTION

SENIOR SCIENTIFIC OFFICER/PRINCIPAL SCIENTIFIC OFFICER (Ref. AG/18/A) (Re-Advertisement)

Applications are invited for the post of Senior Scientific Officer/Principal Scientific Officer to lead a team investigating the effects of agricultural machinery on the physical properties of field soils with particular reference to cultivations, zero-tillage and compaction.

QUALIFICATIONS: A first or upper second-class Honours Degree, and preferably a higher Degree, or equivalent qualifications in a relevant branch of Science or Engineering. The applicant should have had considerable experience of research in soil physics or soil mechanics.

SALARY SCALES: Appointment in the grade of Senior Scientific Officer (£5,154 to £6,898) or Principal Scientific Officer (£6,609 to £8,461) according to qualifications and experience. Non-contributory superannuation.

Application forms and further information from:—

The Secretary, Scottish Institute of Agricultural Engineering, Bush Estate, Penicuik, Midlothian EH26 0PH.
to whom applications should be submitted by October 6, 1978. 7120(A)

CHANGE OF ADDRESS

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EUROPEAN MOLECULAR BIOLOGY LABORATORY EMBL

The European Molecular Biology Laboratory is an international research institute founded by 10 member states (Austria, Denmark, France, Federal Republic of Germany, Israel, Italy, Netherlands, Sweden, Switzerland, United Kingdom) with its Central Laboratory in Heidelberg, Germany and two out-stations in Hamburg, Germany and Grenoble, France.

Applications are invited for the post of

DIRECTOR OF ADMINISTRATION

Applicants should be nationals of the member states, in the age range 45-55 and should have substantial responsible experience in management and preferably of scientific administration. Experience of international organisations would be an advantage. Fluency in at least two of the three official languages (French, English, German) as well as a reasonable working knowledge of the third, is essential.

The successful applicant will be located in the Central Laboratory at Heidelberg and will be offered the basic salary, net of tax, of DM 6300 monthly or higher according to qualifications. Additional allowances are payable according to personal circumstances.

Applications should be addressed to:

Sir John Kendrew, EMBL, Postfach 10.2209, D-6900 Heidelberg, Germany. 7140(A)

TECHNICIAN (HISTOPATHOLOGY)

Qualified to at least ONC level, with the relevant background, to assist in the work of the Histopathology Laboratory of the Division of Viral Products. This involves the preparation and examination of tissues from experimental animals inoculated with human virus vaccines. Although training will be provided, experience in the preparation and processing of tissues and competence in handling laboratory animals would be an advantage.

The post offers prospects for promotion. Salary up to £5,034 depending upon experience and qualifications.

The Institute is situated in pleasant surroundings, close to Hampstead Underground Station, but is expected to move to new premises outside London in 4-5 years' time. Please write, or telephone, for an application form, quoting reference number 0140, to The Personnel Officer, National Institute for Biological Standards & Control, Holly Hill, Hampstead, London NW3 6RB. Tel: 01-435 2232 extn. 211.

7199(A)

NIBSC

National Institute for Biological Standards and Control

UNIVERSITY OF OXFORD DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL RESEARCH ASSISTANT GRADE 1A

(3 Year Appointment)

Applications are invited for a Post-doctoral Research Assistant supported by the S.R.C. and tenable for three years, to work with Dr Peter C. Newell on genetic aspects of development in the cellular slime mould *Dictyostelium*. The project will employ the parasexual cycle of this eukaryotic micro-organism to study the genetic loci involved in the production of a simple morphogen that controls the course of differentiation. The appointment will commence on November 1, 1978, or soon after, on the salary scale £3,660 to £4,601 (under review). Applications, including the names of two referees, should be sent to The Administrator, Department of Biochemistry, South Parks Road, Oxford OX1 3QU by October 28, 1978. 7195(A)

OXFORD UNIVERSITY PHYSICAL CHEMISTRY LABORATORY POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a two-year post to study the binding of a hormone or drug to a receptor site by the method of computer simulation. The project is supported by the Medical Research Council, and the post will be on the 1A Scale at a starting salary of up to £4,130 and with membership of U.S.S. Applicants experienced in computer programming should write for further particulars to Professor J. S. Rowlinson, Physical Chemistry Laboratory, South Parks Road, Oxford OX1 3QZ. 7180(A)

UNIVERSITY OF BRISTOL DEPARTMENT OF PATHOLOGY RESEARCH ASSISTANT (£4,382 to £5,367)

Applications are invited for the above post from biological scientists with experience in cell membrane protein biochemistry, and immunology. The successful applicant will join a new M.R.C. funded 5-year programme to investigate the use of membrane antigens as a possible EB-virus vaccine as part of a wider study; technical help will be available. The appointment will be for one year initially renewable annually. Replies in writing giving the names and addresses of two referees to Professor M. A. Epstein, Department of Pathology, The Medical School, University Walk, Bristol BS1 1TD, by October 6, 1978. 7185(A)

MCGILL UNIVERSITY BIOCHEMISTRY DEPARTMENT STAFF POSITION ASSISTANT PROFESSOR

to teach graduate and undergraduate courses, supervise students and maintain an active independent research program. Preference will be given to the general area of enzyme regulation in mammalian cells. Candidates should have at least two years of postdoctoral experience. Curriculum vitae, including the names of at least three persons who can be contacted for letters of reference, should be sent to:

Dr W. Mushynski,
Search Committee,
Department of Biochemistry,
McIntyre Medical Sciences Building,
McGill University,
3655 Drummond Street,
Montreal, Quebec,
Canada H3G 1Y6. 7203(A)

PLANT BREEDING INSTITUTE Maris Lane, Trumpington Cambridge CB2 2LQ CYTOGENETICS DEPARTMENT SCIENTIFIC OFFICER

A Scientific Officer is required to undertake under the supervision of Dr. M. D. Bennett experimental studies of reproductive cell development in cereals with special emphasis on premeiotic and meiotic chromosome behaviour, and of variation in the amount and type of chromosomal DNA within and between higher plant species, particularly crop plants and their relatives, using a wide range of cytochemical, autoradiographic, light and electron microscopy techniques.

Candidates should have an honours degree or equivalent in biology, chemistry and/or physics; or a postgraduate degree, HNC or equivalent with relevant post-qualifying experience. Laboratory experience in electron microscopy or cytology would be an advantage.

Starting salary will be according to qualifications and experience on scale rising from £2,839 p.a. to £4,415 p.a. Non-Contributory Pension Scheme.

Applications with curriculum vitae and names addresses of three referees should be submitted to the Assistant Secretary (Establishment) not later than 9th October 1978. 7194(A)

TEMPORARY MEDICAL LABORATORY SCIENTIFIC OFFICER IN HISTOPATHOLOGY

Qualified technician required for months only. Hospital experience in Histopathology essential.

Application form from the Personnel Department, Royal Marsden Hospital, Fulham Road, London SW3. Tel: 01-352 8171 Ext. 447. 7187(A)

CHANGE OF ADDRESS

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**UNIVERSITY
OF MANCHESTER
DEPARTMENT OF MEDICAL
BIOCHEMISTRY
POSTDOCTORAL
RESEARCH ASSISTANT
IN BIOCHEMISTRY**

A postdoctoral research assistant sponsored by the Medical Research Council is required from October 1978 earlier if preferred. The work, under the supervision of Jacqueline B. Weiss, involves the characterisation of spatial recognition of new collagen alpha chains and the person appointed will be part of a multi disciplinary team of whom one is an electron microscopist. Experience in collagen biology or biochemistry would be very welcome but experience in general protein chemistry would also be acceptable. The appointment is for three years only. The salary scale is in the range £4,365 to £4,834 p.a. Applications, including curriculum vitae, to Jacqueline B. Weiss, Senior Lecturer, Department of Medical Biochemistry, Medical School, Stopford Building, Oxford Road, Manchester M13 9PT. 7075(A)



CHAIR OF GEOLOGY

Applications are invited for a further Chair of Geology which is to be established during the academic year 1978/79.

Salary within the professional range. Applicants may have teaching and research interests in any field of study appropriate to a University Department of Geology.

Further particulars may be obtained from the Secretary and Registrar, The University, Southampton SO9 5NH. Send applications (10 copies from applicants in the United Kingdom) to be submitted before November 5, 1978. 7130(A)

**THE UNIVERSITY OF THE
WEST INDIES, TRINIDAD**

Applications are invited for the post:

**LECTURER/ASSISTANT
LECTURER IN INORGANIC
CHEMISTRY**

Post is to be filled for Academic year 1979/80. Salary Scales: Lecturer: £19,071 to 29,799 p.a. Assistant Lecturer: TT\$15,480 to 16,974 p.a. (sterling=TT\$4.62). F.S.S.U. Unfurnished accommodation if available 10% or furnished at 124% or housing allowance of 20% of pensionable salary. Up to five full economy passages on appointment and on normal termination. Study and Travel Grant. Detailed application (2 copies) with curriculum vitae and naming three referees to be sent direct as soon as possible to Secretary, University of the West Indies, St Augustine, Trinidad.

Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, LONDON W1P 0DT. Further details may be obtained from her address. 7179(A)

**unilever
research**

Biological Evaluation Scientist

Our Environmental Safety Division has a vacancy for a Biological Evaluation Scientist.

The Division carries out basic research in toxicology and is responsible for the toxicological safety of Unilever Products.

The Scientist appointed will be responsible for the planning and implementation of tests to assess the safety of substances on skin.

Candidates, of either sex, should have a good Honours degree in a bio, biochemical science and three or more years post graduate experience leading to a Ph.D or at an equivalent level. Training or experience in toxicology particularly the skin area would be an advantage, but is not essential.

The Colworth Laboratory is located on a very attractive 1200 acre country estate ten miles north of Bedford. A competitive salary will be offered and an excellent benefit package includes BUPA and assistance with re-location expenses, where appropriate.

Please write quoting Ref. CH-228M and giving details of career history to:

**Miss J. Kenny,
Personnel Officer,
Unilever Research,
Colworth Laboratory,
Colworth House, Sharnbrook,
Bedford MK44 1LQ.**

7172(A)



**UNIVERSITY OF DUNDEE
DEPARTMENT OF
BIOLOGICAL SCIENCES
Applications are invited for a
RESEARCH ASSISTANT**

to work in the above Department under the supervision of Dr M. J. Daft.

The post is financed from an external grant and will be available for up to three years. The investigation is on interactions and competitive effects amongst different endomycorrhizal crop plants. Applicants should have an Honours Degree in Biology.

Salary scale £3,189 to £3,660.

Applications, in writing, containing the names of two referees should be sent to The Secretary, The University, Dundee DD1 4HN. Please quote Ref: EST/58/78J. 7158(A)

**UNIVERSITY OF
NOTTINGHAM
RESEARCH ASSISTANT IN
BIOLOGICAL CHEMISTRY**

To work on the isolation and identification of novel antibiotics from hybrid fungi and actinomycetes. The position which is tenable for two years in the first instance requires a graduate with a good degree in either Chemistry or Biochemistry, preferably with a knowledge of microbiology.

Salary £3,384 per annum (Under review).

Further particulars and forms of application, returnable not later than September 30, 1978, from the Staff Appointments Office, University of Nottingham, University Park, Nottingham NG7 2RD. Ref. No. 630. 7153(A)

DELALANDE Research Center

wishes to recruit an additional research worker in the area of

pharmacokinetics and drug metabolism

Applicants should already be working in this field and should have a PhD or equivalent qualification. Knowledge of French is not essential.

DELALANDE is an international pharmaceutical company actively engaged in research. The Research Centre is situated in attractive surroundings close to Paris and is within easy reach of main road and rail routes.

Applications should be sent to:

M. STROLIN BENEDETTI

Head, Biochemical Pharmacology

Centre de Recherche DELALANDE

10, rue des Carrières

92500 RUEIL-MALMAISON

FRANCE



DELALANDE Research

7200(A)

HAVAS CONTACT

CHANGE OF ADDRESS

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Department of Agriculture and Fisheries for Scotland

Agriculturist a specialised role in potato agronomy

The Agricultural Scientific Service undertakes a wide variety of experimental projects including seed testing, National List testing of new varieties of crops, seed and planting stock certification schemes and the development of plant health standards.

This post offers an experienced agriculturist the opportunity to develop and manage the production and distribution in Scotland of virus-tested nuclear and commercial seed potato stocks under quarantine conditions. The successful candidate will also help to monitor nuclear clones for potato leaf roll virus, will control work on the Department's potato variety and disease collections, plan and organise experimental projects, mainly in physiology and agronomy, and assist in training potato inspectors and specialist agricultural staff. Candidates, normally aged under 32, should preferably have a good honours degree in agriculture, botany or close related subject, and must have a sound knowledge of potato agronomy and physiology and a good appreciation of potato diseases and their control.

Appointment as Senior Scientific Officer (£5150-£6900) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 12th October 1978) write to Scottish Office, Personnel Division, PM(PTS)3, Room 404, 16 Waterloo Place, Edinburgh EH1 3DN, telephone Edinburgh (031) 557 2090 ext 283/248. Please quote ref: SA/17/DLA.

7154(A)

**Science
Group**
CIVIL SERVICE

UNIVERSITY OF LONDON INSTITUTE OF NEUROLOGY

Muscular Dystrophy Research Laboratories, Institute of Neurology, London. Postdoctoral position is available for a biochemist to study cell surface glycoproteins and glycolipids of muscle from a recessive genetic disorder grown in culture. Experience with techniques of surface labelling and isolation of membrane lectins would be of value. Position is available October 1. Starting salary is £4,155 (at age 26) plus London Allowance. Send curriculum vitae and names of three referees to Dr E. J. Thompson, Neurochemistry, Institute of Neurology, London WC1N 3BG.

7150(A)

TECHNICIAN GRADE 2B

required for the Zoology Department to work in the photographic unit and occasionally in other areas of the Department. City and Guilds 744/745, O.N.C., or equivalent qualifications and three years experience required preferably in the above discipline. Day release scheme for further training. Salary on scale £2,994 p.a. rising to £3,345 p.a. (inclusive) 4 weeks annual holiday. Contributory pension scheme. Apply in writing with full details to: The Head Clerk, Ref. 204011/N, King's College London, Strand WC2R 2LS.

7186(A)

A.R.C. ANIMAL BREEDING RESEARCH ORGANISATION ASSISTANT BIOMETRICIAN

Required for research on the genetics of growth in relation to animal production. The work involves analysis of a wide range of data and participation in the conduct of experiments. Applicants should have an honours degree in a relevant subject and some ability in computing. Appointment as Scientific Officer (£2,839 to £4,415) with starting salary depending on qualifications and experience. Non-contributory superannuation. Further information and application forms obtainable from the Secretary, A.R.C. Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ. Closing date October 7, 1978.

7141(A)

PRE AND POSTDOCTORAL POSITIONS

are available for studies on the protein synthesising system with special emphasis on ribosomes—topography, biogenesis and function—under various conditions of growth and development. Teaching opportunities available. Reply with résumé to:

Professor Bruce H. Sells,
Laboratories of Molecular Biology,
Faculty of Medicine,
Health Sciences Complex,
Memorial University of
Newfoundland.

7173(A)

FELLOWSHIPS

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London) JUNIOR RESEARCH FELLOWSHIP

Applications are invited for a Junior Research Fellowship (£1,990 p.a. plus approved fees) tenable for 3 years jointly in the Department of Medical Physics for experimental studies in ultrasonic diagnostic techniques. Applicants should preferably have some experience in computer programming.

Applications, with curriculum vitae and the names of two referees, should be sent as soon as possible to Professor H. A. B. Simons, Department of Medical Physics, Royal Free Hospital, Pond Street, London NW3 2QG.

7190(E)

UNIVERSITY OF WARWICK POSTDOCTORAL RESEARCH FELLOWSHIP

IN THE DEPARTMENT OF CHEMISTRY AND MOLECULAR SCIENCES

Applications are invited for a Postdoctoral Research Fellowship to work with Dr D. W. Hutchinson on the synthesis and properties of oligoadenylic acids as inhibitors of protein synthesis. This work, which is funded by the M.R.C., will be carried out in collaboration with Dr I. M. Kerr of the National Institute for Medical Research, Mill Hill, London. The appointment will be for two years commencing as soon as possible. Initial salary will be up to £5,730 p.a. on the Range 1A scale: £3,660 to £6,178 p.a. Applications, together with the names of two referees, should be sent to the Academic Registrar, University of Warwick, Coventry CV4 7AL quoting Ref. No. 6/2A/78 as soon as possible.

7161(E)

UNIVERSITY COLLEGE

DUBLIN

DEPARTMENT OF CHEMISTRY

POSTDOCTORAL FELLOWSHIP

IN

PHYSICAL

ORGANIC CHEMISTRY

Applications are invited for the above post to investigate the activating effect of the azomethine function implicated in catalysis by the coenzyme pyridoxal phosphate. The appointment, which is supported by the Irish National Board for Science and Technology, will be made as soon as possible and will be renewable annually for up to three years from October 1, 1978.

Initial salary will be in the range £3,762 to £4,388.

Applications accompanied by a curriculum vitae and names of two referees should be sent to:

Dr R. A. More O'Ferrall,
Department of Chemistry,
University College,
Belfield, Dublin 4.

7202(E)

UNIVERSITY OF YORK

DEPARTMENT OF BIOLOGY

POSTDOCTORAL FELLOWSHIP

Applications are invited for an M.R.C.-funded postdoctoral research fellowship to work for three years on a project comparing and contrasting the protein and glycoprotein composition of myelin and oligodendrocyte membranes from normal and multiple sclerosis nerve tissue. Applicants should have expertise in membrane proteins, glycoproteins and related separative techniques.

Starting salary in the range £3,660 to £4,834 (under review).

Applications should be sent as soon as possible to Dr M. G. Rumshy, Department of Biology, University of York, Heslington, York YO1 5DD, and should be in the form of a curriculum vitae with the names of two referees. Further information can be obtained from the same source. Please quote reference number 11/6094.

7182(E)

STUDENTSHIPS

THE UNIVERSITY OF ADELAIDE DEPARTMENT OF SOIL SCIENCE Applications are invited for a RESEARCH STUDENTSHIP

to support research for Ph.D. with A. R. Dexter of the Waite Agricultural Research Institute on an Australian Oilseeds Research Committee project concerned with the interaction between soil structure and root growth of oilseed crops. Tenure of the studentship depends on continuing support from the Australian Oilseeds Research Committee but at least three years support is expected. The stipend payable is \$A4200 per annum plus allowances for dependants. Some assistance with relocation expenses may be available. Applicants should be graduates with a good honours degree in science subject and an interest in or more of the following would be an advantage: plant growth, soil mechanics, geometry and mathematical modelling.

Applications with a curriculum vitae and the names and addresses of two academic referees should be sent to the Academic Registrar, University of Adelaide, G.P.O. Box 498, Adelaide South Australia, Australia 5001. Closing date October 1978.

7198(E)

SHEFFIELD CITY POLYTECHNIC DEPARTMENT OF BIOLOGICAL SCIENCES RESEARCH STUDENTSHIP

Salary Scale: £2,500 to £3,000 p.a.

Applications are invited for appointment as a Research Student to undertake research into the utilisation of fractions of town refuse in land reclamation schemes.

This work will be carried out in conjunction with South Yorkshire County Council. Applicants should possess a good honours degree in Biological Sciences, Biochemistry or Soil Sciences, and the successful applicant will be expected to seek retraining for a higher degree.

Requests for an application form writing only please to the Recruitment Section of the Personnel Department, Sheffield City Polytechnic (Dept. NA Halfords House, Fitzalan Square, Sheffield S1 2BB. Completed forms should be returned by October 6, 1978.

7177(E)

UNIVERSITY OF LONDON KING'S COLLEGE PHYSICS DEPARTMENT

Applications are invited for a Ph.D. research studentship, tenable for three years from not later than January 1, 1979, for experimental research on the production and properties of cold centres in covalent crystals. The value of the studentship will be at least that of an S.R.C. grant. Further details are available from Dr E. Lightowler, Physics Department, King's College, Strand, London WC2R 2LS.

7151(F)

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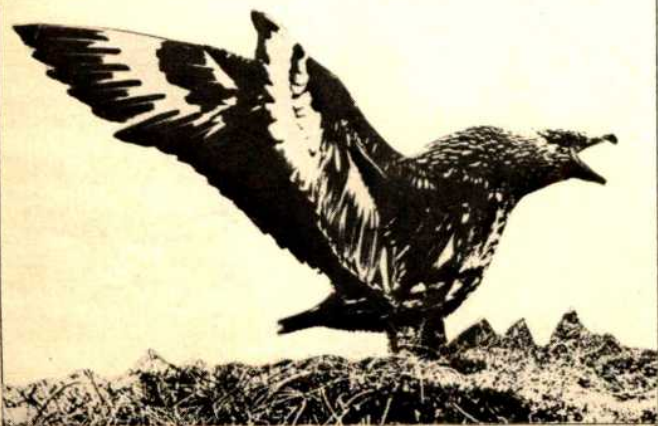
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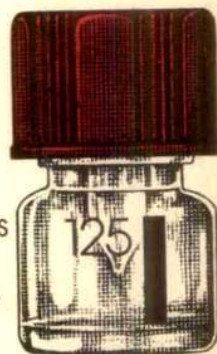


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in aqueous solution

NEZ-033A Low pH 8-10
Medium concentration
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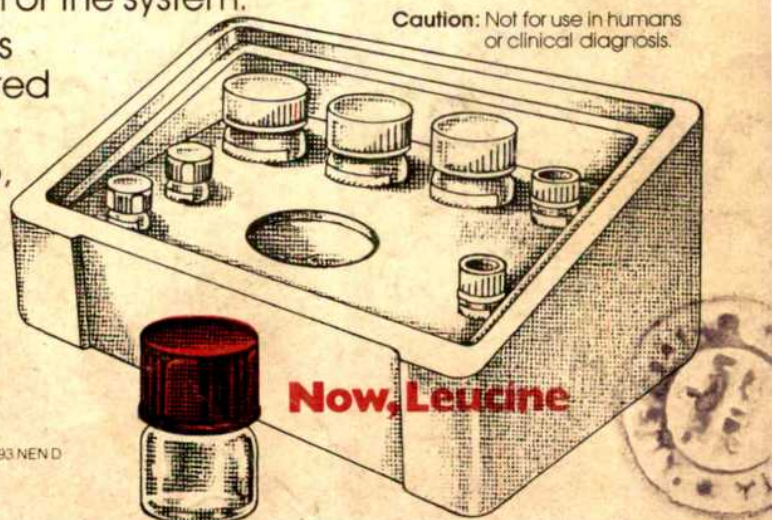
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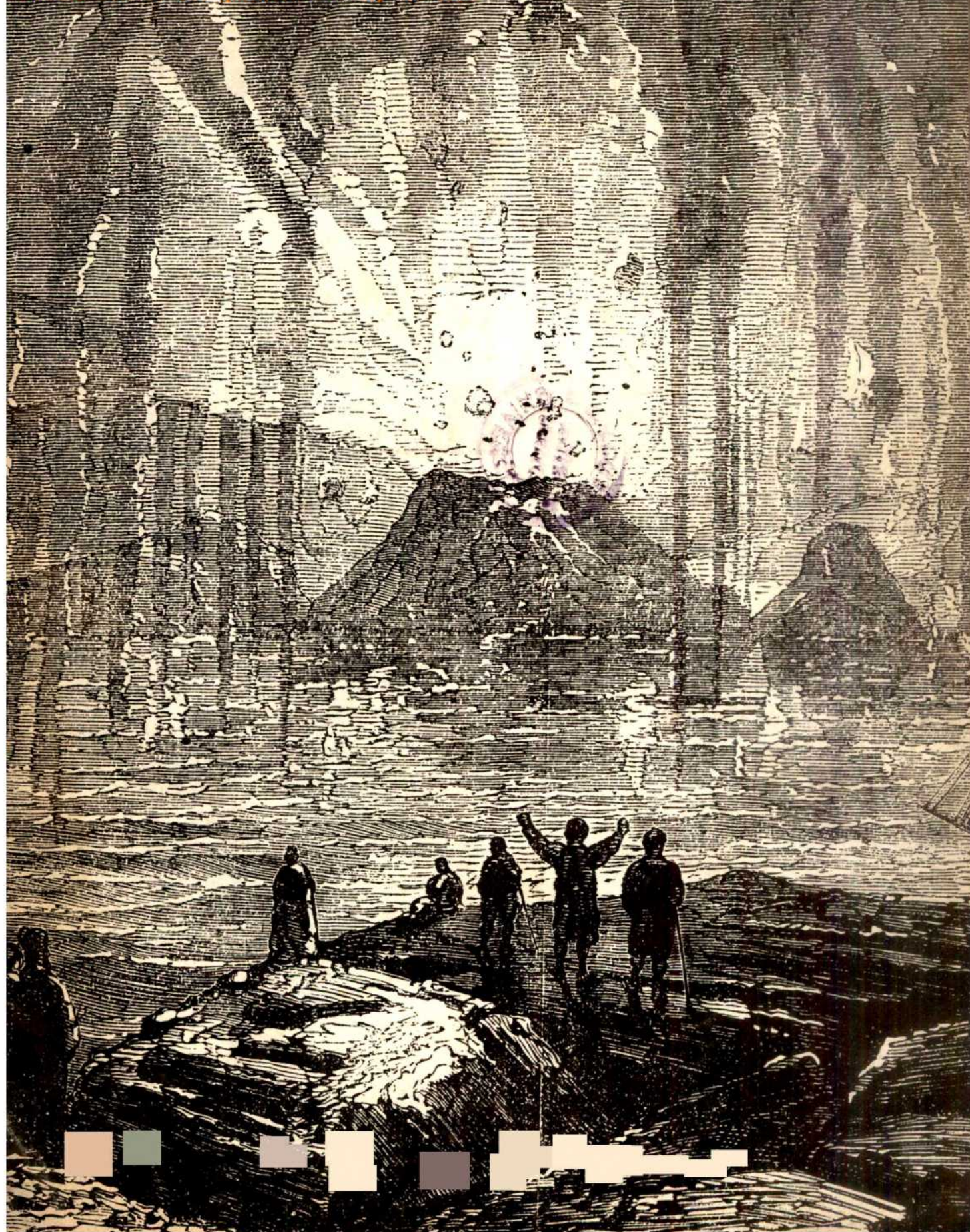
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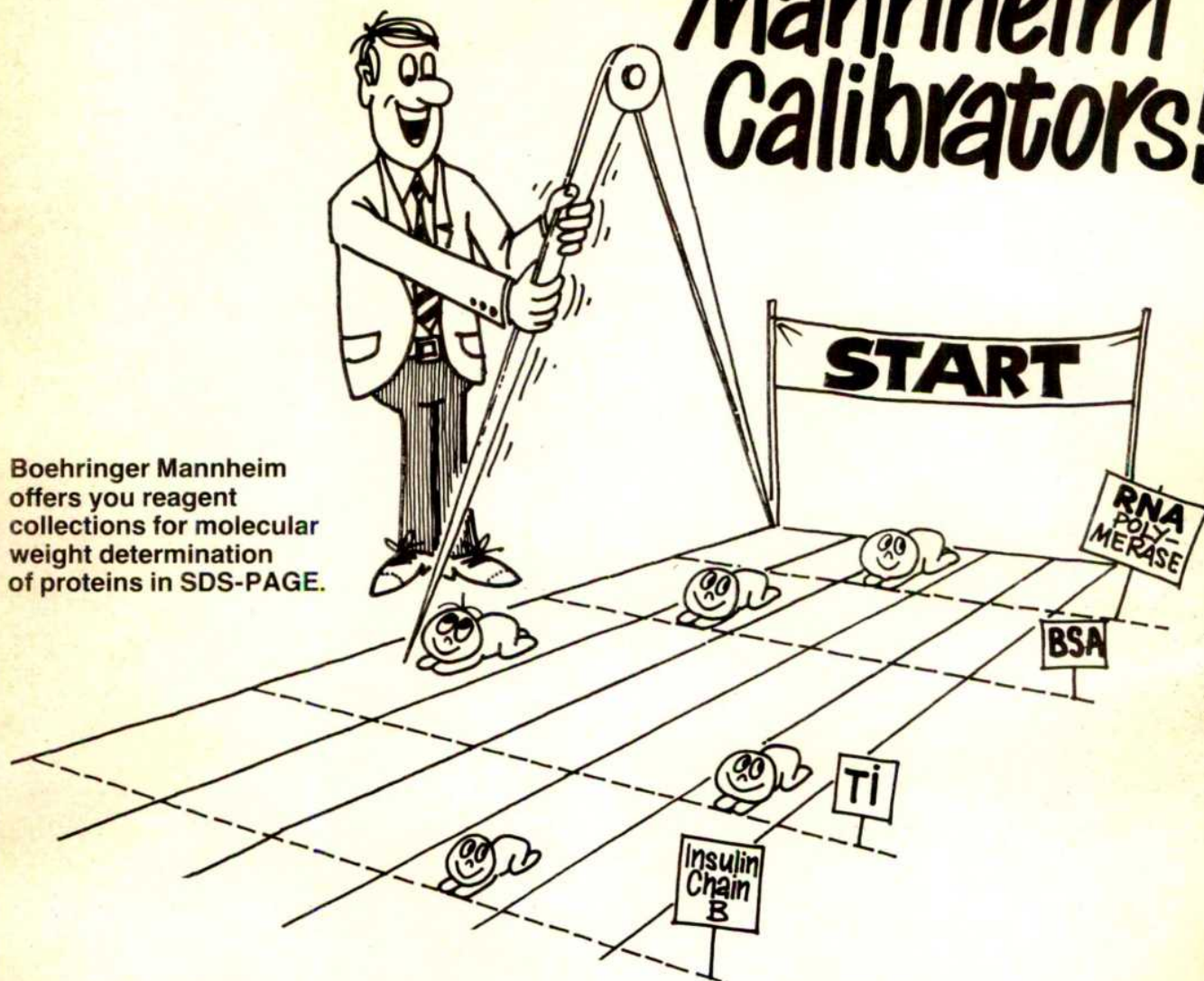
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writes about the recent shipboard
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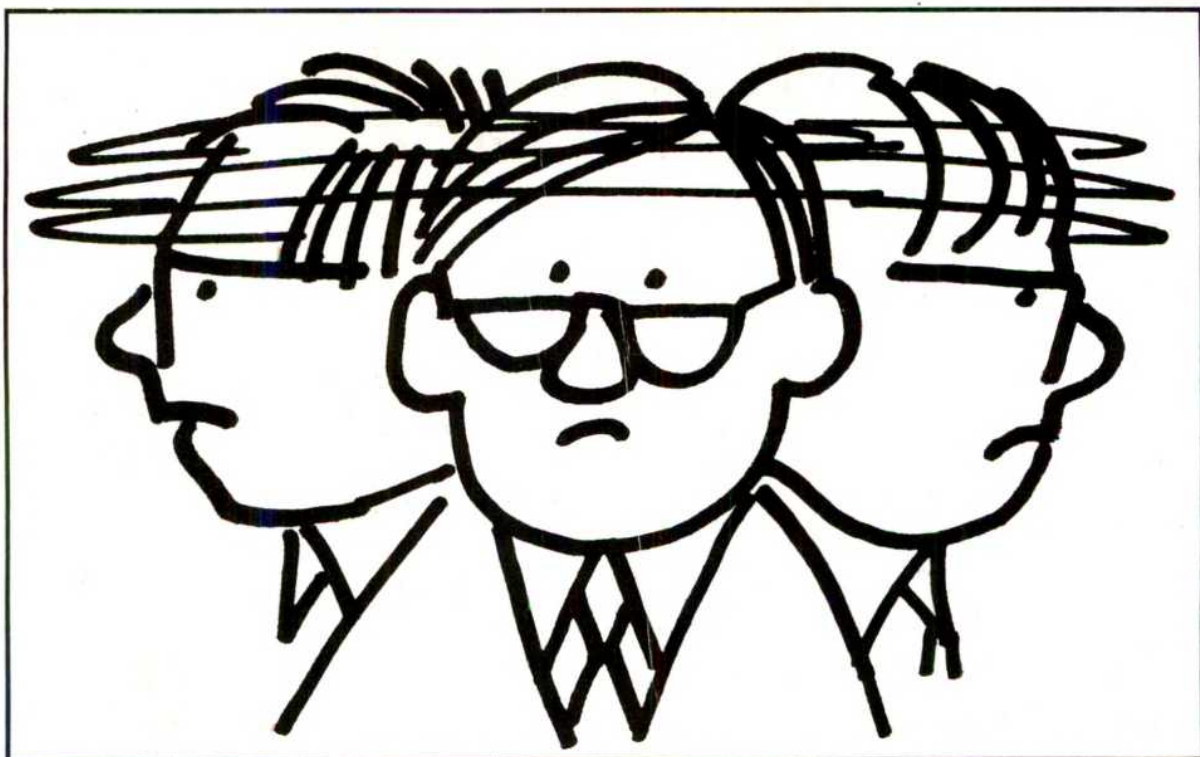
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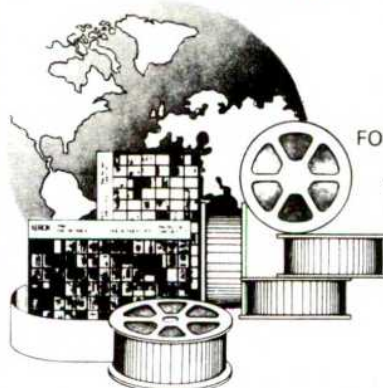
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28 September 1978

To Geneva yet again

REPRESENTATIVES of the United States, United Kingdom and Soviet Union are back in Geneva this week to resume talks on a comprehensive ban on nuclear testing. Regular readers of these columns could be excused for stifling a slight yawn on hearing this; for many years now it has seemed to us and to others that a ban was just around the corner. Each year we have been reliably advised that the odds were fifty-fifty on a treaty by Christmas, but those who believe that this implies ultimate success should ponder the message of the coin tossed in Tom Stoppard's play *Rosencrantz*—which came down 'heads' more than eighty times in a row.

At present the two superpowers operate a voluntary limit of 150 kilotons on underground tests, and they, along with most other countries, are prevented from firing above ground by the Partial Test-Ban Treaty of 1963. France and China, non-signatories of the treaty, used to test regularly in the atmosphere, but in recent years, they too have gone underground. Although only three countries are party to the present negotiations on a comprehensive treaty, it is hoped that if and when a document is produced it will prove widely acceptable, and even those seasoned non-signers, France and China, will be prepared to adhere to its spirit. It goes almost without saying that many nations under strong pressure not to venture into nuclear proliferation are very keen indeed for some evidence that the superpowers are putting a brake on their own weapons programmes, and a comprehensive test ban, although only a partial dampener on weapons development, has great symbolic importance.

What then holds up a treaty? The traditional reason for delay has been the inadequacy of systems for monitoring compliance, whether by seismic or other means. There has been considerable progress in these matters in the past ten years; further major improvements in the near future are, however, unlikely. Above certain yields the risk of detection and identification is now very high, but below these levels there is more and more scope for undetectable clandestine activity. No technical monitoring system can provide complete satisfaction. Fortunately this now seems to be widely understood; no interested party would now be at liberty suddenly to 'discover' inadequacies in the monitoring system and claim that negotiations had been conducted in ignorance of them.

A more recent obstacle to a treaty has been peaceful nuclear explosions. The almost complete loss of support for these in the United States, on economic and public-acceptability grounds, contrasts with enthusiasm within the Soviet Union, where more than forty such explo-

sions have been fired for a variety of purposes. But even in the Soviet Union the pace seems to have slackened off of late, and elsewhere no one—not even the Indians—now has much enthusiasm. The trouble with peaceful explosions is that there is absolutely no way of verifying that they do not also have a military purpose, and any attempt to exclude such explosions from a comprehensive test-ban would have to be viewed with the utmost suspicion.

Most recently, concern has been expressed in the United States about the effects that a test-ban would have on stockpile efficiency; it is claimed that in the absence of regular testing, devices cannot be trusted indefinitely. This is a controversial matter; not even all those associated with the weapons laboratories would have the same point of view. But there have already been calls for the ban not to be total, but to allow limited low-yield testing. If peaceful explosions could be a vehicle for weapon development, however, so too could ostensible stockpile testing.

So what will emerge from Geneva? It has to be acceptable to the US Senate which ratifies such international agreements, so it will probably err on the side of caution. The Soviet Union seems prepared to forego peaceful explosions, but only for a short period. A limited duration treaty would suit the United States fine, because it would sidestep the question of the future of the weapons laboratories. At a guess there will be a three year ban on the firing of any nuclear devices whatsoever. But do not be deceived; although the treaty will undoubtedly come up for renewal there must be the most serious doubts that it actually will be renewed. Between 1958 and 1961 there was a thirty-four month moratorium in testing but it did not lead to a permanent ban. From public pronouncements in the United States and from an apparent Soviet desire to push ahead on the peaceful uses front, it seems clear that many have already made up their mind that the ban will merely be a temporary measure.

What purpose does a British presence serve at these talks? Undoubtedly it has a certain cosmetic value in dispelling the impression that the two superpowers are colluding, and probably there are technical matters where British representation injects a sensible new dimension. But Britain is so closely tied to the United States in nuclear affairs that it is most unlikely that a truly independent line could be taken. It is probable that the treaty which will emerge from Geneva will be a poor substitute for a truly comprehensive permanent ban; somewhere an independent powerful and respected voice should be saying this. □



Sinai in peace and in war: one plan was to make it an Arab-Israeli "national park"

Israel wants scientific cooperation with the Arabs

ISRAELI scientists see the outcome of the Camp David talks as a unique challenge and chance for mutual cooperation—a result which may, at first, seem paradoxical, since the settlement will mean handing over to Egyptian control one of the most strictly protected conservation areas in the world.

During the 12 years that Sinai has been under Israeli control, environmental restrictions have been so strict that not even a single shell could legally be removed from the beach without special permission. The fragile ecology of the area has been extensively studied, and investigations of the "solar lake", a few kilometres south of the pre-1967 frontier have provided a new concept in the commercial use of solar energy. For this lake has a natural stratification of salinity allowing bottom water temperature to build up to more than 60 °C, without convective heating of the upper layers which remain at approximately 20 °C. Not surprisingly, at the international conference on the application of solar energy held earlier this month at the Technion—Israel's institute of technology—there was a whole session devoted to artificial solar ponds, which, it is hoped, will ultimately be used for electricity generation, desalination of sea-water and for low temperature industrial heat production.

As far as the ecology of the Sinai is concerned, the non-governmental Society for the Protection of Nature, which has maintained three field study centres in the Sinai—at Na'ama, Yamit, and Santa Katarina—is willing to extend to the Egyptians any assistance they may require. Indeed, Professor Amotz Zahavi told *Nature* that several years ago, the society suggested that the Sinai should be made into an "international park for peace" under joint Israeli/Egyptian control.

For some years, said Professor Zahavi, the society has been passing on its findings and ideas on conservation to the Israeli authorities. Now, with the hand-over to the Egyptians, their experts would be willing to stay on at the field centres, working in collaboration with the Egyptians for the sake of conservation. Professor Zahavi stressed

that the Israeli teams have no idea how the Egyptians will react to this proposal. "So far as we know, conservation is not yet very well developed in Egypt," he said, a further reason why his society is so concerned to help.

Israel has an impressive record in desert reclamation and the cultivation of marginal lands. With perhaps one-third of the world's cultivable land threatened with desertification, Israeli environmentalists describe as tragic the Arab walk-out from last year's conference on desertification when an Israeli delegate rose to speak.

At Haifa, the Kogan-Rose desalination pilot plant, which uses a counter-flow system to eliminate the need for expensive copper-tubing heat-exchangers, has proved its worth, and plans are underway to construct a full-scale installation capable of delivering some million cubic metres per day. The main question to be decided now is where to build it. While such questions are, of course, matters for the politicians to decide, Professor Abraham Kogan, the designer, speaks of a possible joint Israeli-Egyptian venture.

Indeed, the consensus of Israeli scientific opinion seems to run ahead of the formal political situation. Last summer, in his report to the international board of governors of the Technion, made on the occasion of the 30th anniversary of Israeli independence, the Technion president, Amos Horev, stressed that technological co-operation "could well be put into effect with Arab neighbours even in advance of formal peace". Ecologists and technologists are already looking hopefully towards other frontiers. The Dead Sea potash works, for example, which produces fertilizers from evaporated Dead Sea brines, must use a certain amount of non-saline water in the extraction process. With a growing world demand for fertilizer and a virtually inexhaustible supply of raw material, it is small wonder that some engineers think longingly of the fresh water supply, just across the Jordanian frontier, which in British Mandate times was actually piped to the forerunner of the present potash works (then sited at the northern end of the Dead Sea).

Jordan is known to be considering a potash works of her own—a joint development would be more economical and also a powerful factor in the continued peace of the area.

Likewise, marine ecologists in the Gulf of Aqaba stress that however carefully they monitor the tankers discharging at the Eilat oil terminal, the gulf is narrow, and has no real tidal scour. A joint protection and monitoring programme involving all the littoral states—Israel, Jordan, Saudi Arabia, and Egypt—is the best hope of avoiding major pollution in this part of the gulf.

And, looking even further ahead, Professor Y. Avnimelech of the Kinneret water research project even mentioned the old Johnstone plan of Mandate times, by which the river Yarmuk would be diverted into Kinneret, the augmented waters of which could then be used for the benefit of what now constitutes both Israel and Jordan. Since the Kinneret now serves as the sole fresh water source for Israel's national water grid, and the Israeli water economy is so tight that brackish and waste water irrigation is an increasing necessity, even the most academic allusion to such a scheme reflects a considerable commitment to the idea of cooperation.

Such a commitment, however, is more than a pipe-dream of a few scientists, however far ahead the realisation may be, the Israeli government undoubtedly endorses the underlying principle. Addressing the international congress of ecology, which was held in Jerusalem during the week of the Camp David talks, Mr Yigal Yadin, the Israeli deputy prime minister, stressed that "ecology has no national boundaries". After outlining Israel's considerable achievement in developing the country without major ecological damage, while at the same time extending aid to such third world countries as would accept it, he concluded—"We are yearning for peace so that we can strive for a better world. Let us endeavour when peace comes on earth—if the earth still be there—we may be more worthy to enjoy it".

Vera Rich

Argentina: concern over continued disappearances

On the eve of the Twelfth International Cancer Congress in Buenos Aires, **David Dickson** reports on the fate of 15,000 missing Argentinians, many of them scientists

Two weeks ago Dr Lepanto Bianchi, an Argentinian orthopaedic surgeon, was arrested and taken from his office by men who claimed that they were from the federal police force. Dr Bianchi's arrest follows the similar disappearance last month of Beatriz Perioso, president of the Argentine Federation of Psychologists, who was taken away from a child care centre at which she had been working.

No news has since been received of Dr Perioso's whereabouts, nor whether any charges have been made against her. And neither has any information been received about two other psychologists, Arturo Smith and his wife Celia Kriado, who were taken from their home the following day.

The American Psychological Association has written to the Argentinian President, General Jorge H. Videla, requesting information about the situation of the three psychologists, but no reply has been received.

Professor freed

Meanwhile, however, the Argentine authorities last week released Dr Claudio Bermann, a former professor of psychiatry at the University of Cordoba and director of a psychiatric clinic, who had been arrested and held without specific charges since April 1976.

A number of foreign institutions, including Amnesty International and the US National Academy of Sciences' Committee on Human Rights, as well as the Buenos Aires-based Permanent Assembly on Human Rights, had pressed the Argentine government for his release. Last week he was given permission to leave the country, and flew directly to Israel.

In August Elena Sevilla, a research physicist at the Universidad del Sur in Bariloche who had been arrested in November 1975 in hospital shortly after giving birth, was allowed to leave the country after similar moves had been made on her behalf and is now in the US. However in the same month the Permanent Assembly was notified of 11 more "disappearances", and suspects the actual figure may be four or five times this number.

A number of scientists are planning to raise the issue of human rights at the Twelfth International Cancer Conference, which begins in Buenos Aires next week, and is expected to be attended by 7,000 scientists from all over the world.

The National Academy of Sciences, following a visit to Argentina earlier

this year by three representatives of its committee on human rights, decided not to endorse a proposed boycott of the meeting by US scientists. However the committee agreed to assist "those Congress attendees who so desire to express their human rights concerns while in Argentina," primarily through supporting the activities of the human rights clearinghouse of the American Association for the Advancement of Science.

A number of those attending the conference are planning to hold regular caucus meetings on the human rights situation in Argentina. And facilities are also being made available to provide conference delegates with information about the current situation by the Permanent Assembly.

Conference officials point out that the high expected number of participants indicates that moves to boycott the conference—a parallel conference, for example, is being held in Paris under the sponsorship of Professor Andre Lwoff, and is expected to attract several hundred participants—are likely to have relatively little impact on the Buenos Aires arrangements.

Other Argentine scientists have publicly objected to the "pernicious propaganda" which they claim is being spread about the current situation. "At present, the universities and research institutes are places of quiet and fruitful work, in contrast to the agitation, the activism and the persecution of professor and scientists that were so frequent before March 1976 [the date of the military coup]", according to two members of the Buenos Aires National Institute of Medicine in a recent letter to science.

Academic asphyxia

The Permanent Assembly, which brings together various Argentinian groups concerned with human rights, sees the situation slightly differently. "In the national universities, if a climate of perfect order rules, it has been achieved at the cost of the virtual extinction of all interchange of ideas, and similarly the elimination of the whole of a vast cultural sector whose political ideas do not coincide with those of the university authorities," the assembly says in the most recent report on its activities.

"This situation has brought about a noticeable impoverishment of the academic level, generating a dangerous climate of academic asphyxia," according to the assembly's third commission,

which is responsible for the areas of culture, religion, the arts, the sciences, the professions and technical fields.

Earlier this year the assembly published in the Buenos Aires newspaper *La Prensa* a list of 2,500 names of individuals who had disappeared since March 1976, and whose current whereabouts were unknown. A further 500 cases were reported to the assembly after the advertisement appeared, and officials estimate that they have only been informed of one-fifth of those who have disappeared, giving a total number of 15,000.

Many killed

It is feared that up to half of these—men, women and children—have been killed. In addition, it is estimated that there are 8,000 political prisoners being held in Argentine goals, at least half of whom have not been through a proper judicial process.

Scientists figure prominently among those who have disappeared or been imprisoned, particularly those previously involved in organisations such as the now-defunct Argentine Physical Society, which had taken an active part in debates on political issues in the period immediately before the military coup of 1976. However, other scientists who have disappeared or been imprisoned have, it is claimed, played little part in political activity.

Over 2,000 scientists, including both university teachers and research workers, lost their jobs when the military government came into power. Many have since left the country: "the low salaries, added to the lack of security, threats, and the lack of an adequate scientific programme, has motivated the exodus of a great number of scientists and professionals," according to the assembly's report.

Some Argentinian scientists who remain are also concerned at the low priority given to education in the national budget. "In the early 1960s, expenditure on education represented about 20 per cent of total government spending; now it is down to 8.3 per cent," according to Professor Jose Westerkamp, a nuclear physicist at the University of Buenos Aires.

Some areas of research, such as nuclear engineering, still flourish. But in many fields of more basic science, the combined lack of professional manpower, adequate funds, and postgraduate students has resulted in a dramatic decline in activity. □

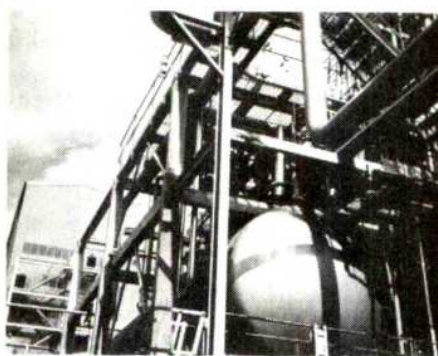
US scientists in new row over cancer at work

PREVIOUS studies of the incidence of cancer associated with occupational exposure to toxic substances may have seriously underestimated the true extent of the problem, according to a statement issued last week by three of the US National Institutes of Health.

Such studies had tended to estimate that the incidence of cancers which would be associated with occupational exposure was between 1 and 15 per cent. But they had been flawed by factors such as the failure to take into account the latency period of several decades that may occur between exposure and the appearance of a cancer, or the synergistic effect of exposure to several carcinogens, the statement claims.

Extrapolating from the results of recent studies of asbestos workers, it concludes that "there is nothing in the gross cancer statistics for the US population which is inconsistent with the hypothesis that up to 20-40 per cent of all cancers are (or will be in the next several decades) attributable to occupational factors".

Those who have contributed to the statement include Dr Arthur Upton, director of the National Cancer Institute, and Dr David Rall, director of the National Institute of Environmental Health Sciences, as well as



PVC production: dispute over hazards

eight other scientists from these two institutes and the National Institute for Occupational Safety and Health.

To offer a figure for comparing the risks from exposure to various industrial compounds, the statement chose "excess cancers" associated with each. (This quantity is defined as the product of the exposed population, the age-adjusted incidence of cancer and an adjusted risk ratio.) The number of annual excess cancers associated with asbestos is then 13,900, but several other substances have excess numbers almost as high: arsenic, 7,300; benzene, 1,400; chromium, 7,900; nickel oxides, 7,300; and petroleum products, 9,100.

The statement predicts that in the years ahead, cancers caused by exposure to other substances will also begin to rise sharply. "The five other agents together pose hazards similar to or greater than those posed by asbestos," says the report. "If only one of the thousands of chemicals introduced into commerce in the past 30

years proves to be as hazardous as asbestos, this could suffice to maintain comparable rates of occupationally-related cancer for decades into the future."

Several industry scientists refused to comment on the report until they had had several weeks to analyse the data. However the President of the Society of the Plastics Industry called the statement "an embarrassment to medical science". He cited a section of the report that predicted 1,940 excess cancer deaths a year to exposure to vinyl chloride as evidence of faulty methodology. "The facts show clearly that vinyl chloride is hazardous to man only at high levels of exposure and over relatively long periods of time", he said. The levels of population considered at risk by the statement are much too high, he concludes, saying that only 23 cases of cancer caused by exposure to vinyl chloride have been confirmed in the last 16 years.

The statement, which is based largely on a number of individual studies carried out by various of its authors, was used by Health Secretary Joseph A. Califano as the basis of a speech earlier this month to a national conference on occupational health organised by the American Federation of Labour-Confederation of Industrial Organisations (AFL-CIO).

In his speech, Dr Califano said that the results were "alarming", and that they would lead to an increased emphasis by the administration on the prevention rather than the cure of cancer.

John Douglas

Radio back-up in rural health service

A VHF communication network is now operating in the rural area of Ballabgarh, 35 kilometres south-east of Delhi. The project is an experiment to see whether such a system is an economic and effective way of providing health care for India.

The network was planned and set up by the Information, Planning and Analysis Group of the Electronics Commission for the All India Institute of Medical Sciences which is running a Comprehensive Rural Health Service Project at Ballabgarh, providing health services to the local community and teaching medical and paramedical staff.

The VHF network consists of four 15-watt fully transistorised transceivers—three fixed and one mobile—and one 400-mW walkie-talkie set. The fixed transceivers are at Ballabgarh Referral Hospital and two primary health centres and the mobile transceiver in a hospital van. The walkie-talkie set is provided to health workers during visits to patients in villages to communicate

with the main hospital. The network covers 368.64 square kilometres and a population of 100,000.

In India, where the majority of people live in villages, health centres are widespread with no telephone links between them. Information about both routine and emergency cases has to be delivered by hand and may often arrive too late to be of any use. A communication network between health centres is therefore seen as essential for effective health care in rural areas, and a VHF communication network is a novel move. The cost-effectiveness has yet to be established. For this purpose, each call made or received from a station is recorded and medical case histories connected with each call pursued to find out the usefulness of the network in treating the patient. If the project proves its worth, its scope could be enlarged and the Ministry of Health and Family Welfare will be asked to establish similar networks in other areas.

Zaka Imam

Scepticism over Soviet promise to let Levich go

FRIENDS and colleagues of Veniamin Levich have greeted with guarded enthusiasm the news that he may shortly receive a visa to emigrate from the USSR to Israel. This is not the first time the 61-year old Academician has been promised an exit visa.

This time, however, the situation seems more auspicious. The announcement of a visa for Levich and his wife, as well as for 17 other families of Refusniks, was made by US Senator Edward Kennedy after his recent visit to Moscow, during which he had talks with Mr Brezhnev.

It seems likely that the current promises are designed to alleviate western reaction to the recent wave of 'human-rights' trials in the Soviet Union. Senator Kennedy himself stressed that the granting of the promised visas would improve international relations. Until the Levichs actually arrive in the West, however, the possibility still remains that this promise is nothing more than a diplomatic gambit in the cause of paper detente.

Vera Rich

Science: a means of locating resources

SOMETIMES I fear that we may lose sight of a few realities when we relate science and technology to development. We must be a little guarded, in case too much of the burden for economic development and advancement of countries be placed on the shoulders of science and technology, as a result of which it will be blamed for the failures which will inevitably occur. Science and technology cannot provide, in themselves, the motivation necessary for development. This lies with the political will and in the social drive of the individuals and communities of a country, both in recognising their opportunities and realising them.

In demonstrating that science and technology are nevertheless critical for development, I will limit my comments to economic development. This is not to ignore the social and cultural aspects, or minimize their significance, but because they require a rather different approach. Nor am I concerned, herein, with the current questioning of the value of economic development, as this can too easily generate only social palliatives instead of pragmatic solutions to poverty and inequity, and result in calls for the simple redistribution of wealth at the cost of increasing it overall. It is a separate issue.

The purpose of economic development, is, by and large, the increase in the production of wealth; and the production of wealth takes place by the application to natural resources of man's labour. Under natural resources, I include not only all natural phenomena and associated resources, renewable and non-renewable, transformable and non-transformable, but also the land itself. These are not the product of man. Labour, in this context, is the effort by man which arises from the application of his hands, brain and the tools which his brain has shown him will increase his efficiency. Thus, wealth is the product of man and his resources, which, obvious as it may seem, is often forgotten, as one too frequently reads of natural resources or human resources being regarded as wealth by themselves. The same applies to the scientific and technological capacity of a society. The point is important because loose thinking on it confuses the issues of the production and distribution of wealth and leads to a loss of objectivity in economic development and the assistance which



By
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science and technology can offer it.

The relationship of science and technology to the production of wealth can be seen once it is recognised that a natural resource is no use until it has been found, described and understood. Science, to an increasing degree, is the identifying process in bringing resources into the production of wealth. Technology, however, is related to labour, and the level of technology possessed by labour is the index of its efficiency. Given unlimited natural resources, wealth may be increased without improvement in technology, but with limited natural resources technology must be improved.

This provides a degree of distinction between many developed and developing countries. In general, the former have increasingly to develop their technological capacity, whilst the latter have, for the most part, still to ensure that their natural resources have been identified scientifically and fully realised economically.

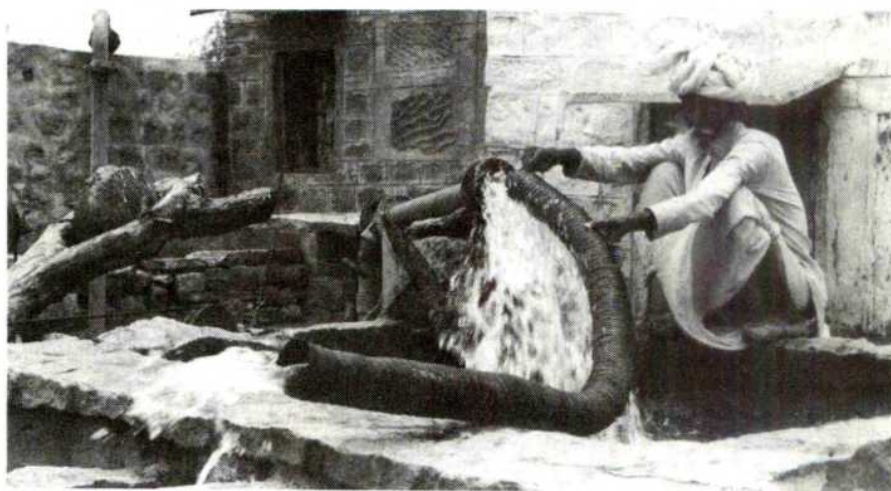
It is important to determine, therefore, if the balance demanded between science and technology is, for any country, the right one in relation to its stage of development and knowledge of its resources. There is a real danger that the cart is being put before the horse: that the developing countries are trying to launch into advanced technologies without an adequate economic justification, when they should still be seeking to upgrade their scientific capacity for its tasks both

of inventory taking and application to renewable resources. Technology is a requirement of circumstances, not an economic paracea. The 'technological gap', as it is called, is as much a reflection of the different needs of countries as it is of restrictive practices, justifiable or otherwise.

The situation must also be seen within the time and circumstances within which it occurs. It has been noticeable that, particularly in countries which in the last decade or so have divested themselves of colonial rule, there has been a decline in the output of the scientific establishments responsible for natural resource inventories and improvement of the yields from renewable resources. This has arisen because of the need of these countries to establish or strengthen their capacity to train their own scientists.

This is a major task, the priority for which has inevitably drawn scientific and technical manpower into the universities, often with better conditions and facilities than exist in the much older applied science establishments. Only now does one see the newly-trained manpower beginning to refill them. It will need more time before able scientists will seek opportunities in industry and only then if its productive capacity and size will sustain viable research and development departments. It is at this stage that the transfer of advanced technologies will really become significant, not only because of the need, but also because there will be an adequate indigenous scientific capacity not only to seize and adapt it, but also, and perhaps more important, to create it.

I think, also, that it is a well to remind ourselves of the basic differences between, and the origins of, science and technology. There seems to be a tendency for administrators, in particular, to regard them as being



Resources: science discovers them, technology applies them

This is a shortened version of a paper presented to a recent meeting on UNCTD convened by the World Federation of Scientific Workers. The full paper will appear, along with others, in the Report and Documentation of the conference currently being prepared by the Federation.

largely similar in quality and thus to be mutually interchangeable and competitive on the same terms. The development of the so-called applied sciences has further tended, in providing a link between the two, to blur the issue. The result has been, I feel, an over-emphasis on the need for science to justify itself in the same way as does technology, and to have an immediate, direct and finite impact on socio-economic problems. Many scientists have subscribed to this, even if unconsciously, in the search for support for their activities. This too often leads to a shortfall in promises regarding its utility in the eyes of administrators and further strengthens their doubts about the efficacy of science compared to technology.

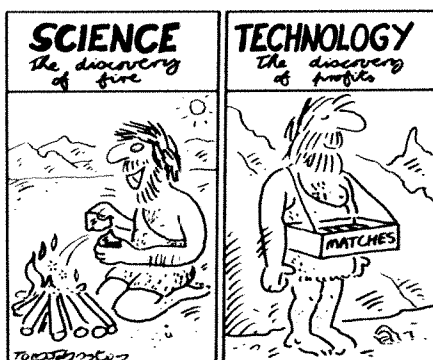
Science, or demonstrable knowledge, is open-ended and has a separate existence. It can be pursued for its own sake, having a cultural value as deeply felt and satisfying as that resulting from the study of art or the humanities, while still serving to relieve man's condition. Technology, in its usually accepted sense, has no existence separate from the material production in which it is involved or for which it was created. Science starts with man's basic curiosity about his environment; whereas technology starts with man's basic economic urge to carry out more easily or profitably a productive activity.

Science, almost by definition, is non-existent until it has been communicated (transferred) to others. Technology has its proof in its products and, therefore, needs not itself to be transferred, thereby conferring rights to its personal ownership and a truly commercial value. Its 'excellence', if I may apply the term, is self-evident. Science, however, needs to be patronised, because its value and excellence is not readily apparent, particularly to administrators. They have to monitor its achievements and excellence, particularly in its more basic aspects, through scientists; and this has, and always will be, an act of faith on their part, which is not a basis for resource allocation they like, particularly since the demand has reached the level it has, today.

Science and technology have been joined in modern times only because much advanced technology has to be science-based; it is now more often manufactured in research and development departments than invented by simple intelligence and manual skill. This, however, does not alter the nature of science and technology, although it does increase the role of science in the production of technology and further emphasises the need for its development.

This brings me to my main point for developing countries, which is that in seeking economic and technical inde-

pendence, particularly in decision-making, they must strengthen their scientific and engineering cadres, not only in their numbers and disciplines, but also in their excellence and problem-solving ability, which latter is the first step to the provision of a genuine indigenous technological capacity. This requires the dynamic teaching of science from the secondary levels of education, through inspired guidance of senior students at the university level, to strictly disciplined research work at the post-graduate level. It means fostering scientific societies, both learned and professional, and the establishment of a stable corps of scientists large enough to be viable and self-critical. It involves increasing considerably the number and quality of



the technical cadres, because most developing countries are characterised by poor ratios of technicians to scientists, which means that any technological improvement or change the latter may prescribe cannot be widely applied.

All this takes a long time and there is no short cut, least of all that based on too early attempts to introduce technologies for which the indigenous technical capacity to handle it is inadequate or absent. We have already seen the mistakes resulting from trying to introduce the applied sciences before the basic sciences are adequately established. To repeat the error in respect of advanced technologies is a greater mistake and can mean an even more extended thralldom, through expensive patents and licenses, to technologies created originally for developed countries.

Moreover, if it is the contention of many developing countries, as it seems to be, that many of these technologies are inappropriate, for whatever reason, technical, economical or social, then it is an even more self-defeating choice as a way to indigenous development. And, it carries with it the threat of limiting the resources for indigenous scientific effort and, thereby, the exploration for, and discovery of, the natural resource potential of a country, which again brings me back to the point I made earlier, namely, that I

believe many of the developing countries need to complete their natural resources inventories before launching into an era of high technology.

I feel that for the realisation of UNCSTD's primary purpose, which is the strengthening of the indigenous technological capacity of countries, that we must think not only of the problems of the choice and transfer of technology but also of the establishment of the right balance between science and technology for development of any country.

Although Unesco's own responsibilities are primarily concerned with the promotion of, and international cooperation in, the natural sciences, whereas other UN agencies have the prime responsibilities for these in the applied sciences, Unesco has increasingly emphasised the important role of natural sciences in relation to the identification and development of the natural resources of developing countries. It is still concerned about the inadequacies both in policy-making in this field and in the creation of effective manpower resources to implement the policy.

In respect of the transfer of technology, Unesco recognises that the problem is much more complicated than that posed solely by the difficulties of gaining access to a particular technology. It involves the creation of a technologically-minded community, not only at the higher levels of specialisation but also at the lower levels of comprehension, where standards and excellence of technical handling are essential for the development of a genuine indigenous capacity which can respond to the needs and opportunities presented to it. Without the development of this basic ability, the transfer of technology alone will remain unfruitful in the long run and tend to separate the communities involved.

For both these reasons, Unesco still stresses the importance of the role of education as one, at least, of the technical prerequisites for strengthening the scientific base of a country and for the successful importation and application of technology. It is, of course, not the only prerequisite. There are social and environmental constraints involved also in the transfer of technology and Unesco has developed programmes in these two fields to examine them and discuss the socio-cultural conditions likely to foster indigenous development processes.

Unesco's activities in this matter of science and technology for development, however, are limited by the availability of funds and the degree of international cooperation it can obtain. We hope that UNCSTD will urge the Member States to be more generous and far-seeing in both these. □

news and views

A massive particle conference

from Frank Close

THE most massive subatomic particle ever discovered was found at Hamburg just one week before a thousand physicists from all over the world gathered in Tokyo for the biennial conference on High Energy Physics. It is the first time that this series of conferences, which began in 1951, had ventured outside of its USA, west-European, east-European cycle. The excitement that has been generated by the discoveries of the last four years has created much cultural interest in the Far East in this most fundamental field of physics, and a delegation from the People's Republic of China was attending such an international gathering for the first time.

The message from the conference was clear. The rapidly accumulating data of the past two years have confirmed our suspicions as to the picture on Nature's jigsaw. The pieces have begun to fall neatly into place and an elegant portrait is emerging.

Unifying the natural forces

The electromagnetic and weak interactions are indeed unified in the "simplest" way, the $SU_2 \times U_1$ model of Glashow, Weinberg and Salam. Briefly, there is much evidence supporting this, and no high energy data that go against it.

To accomplish this unification, Nature has a set of fundamental fermions (quarks and leptons) and has ordered them into left-handed pairs. For leptons we know now that three such pairs (or "generations") exist (*News and Views* 271, 406; 1978): electron and neutrino; muon and a second neutrino, τ and a third neutrino. The existence of the third pair has been firmly established only in the last year. For quarks two generations are well-established. The up and down quarks (found in the proton) have been known for many years, whereas the second generation of strange and charm quarks were established when charmed particles were discovered just before the previous conference in this series (*News*

and *Views* 262, 537; 1976).

Evidence was presented to the conference that a fifth quark (bottom, b) exists. A sixth quark is confidently predicted to complete the third generation of quarks. There is optimism that the advent of PETRA, the new electron-positron colliding beam facility to be opened in Hamburg this month, will provide evidence for this sixth (top, t) quark before the next conference in this series.

The leptons interact by exchanging photons (familiar QED) and also W^+ , W^- and Z^0 "weak gauge bosons". These must be found to really confirm the model. They are predicted to have masses around 70-90 GeV and so we have to be patient for about three years. A session on future accelerators discussed the new machines under plan that may find these objects, and also reviewed the ideas for machines to be built around the world in the longer term and the profound questions that may be asked of them.

The quarks also interact with the above bosons. In addition, quarks carry a quantum number called "colour". New evidence supporting this was presented at the conference: τ decay rates; electron-positron annihilation rates at low energies from Frascati and DCI, Orsay, and the production rates for lepton pairs in proton interactions etc. The quarks can then interact by exchanging coloured gluons. This leads to a theory called "quantum chromodynamics" or QCD (these gluons of QCD are analogues of the photons of QED), and as such provides a theory of the strong (nuclear) interaction.

One consequence is that the scaling phenomenon seen previously in deep inelastic scattering of leptons on proton targets should be violated in a systematic way. In 1976 qualitative support for this prediction was known. It appears that quantitative support is at last emerging now that the older electron and muon scattering data are being supplemented by neutrino interaction data at Fermi Lab. and, in

particular, at the CERN SPS in Geneva. This facility became operational in 1976 and the high precision data that it has provided have been instrumental in establishing the weak-electromagnetic unification and are now testing the QCD theory of strong interactions.

Although the emerging data in lepton and in hadron interactions are all consistent with QCD, no conclusive tests that would confirm or deny the theory are yet available. Theorists are still uncovering some of the rich subtleties in the theory and there was much discussion as to the understanding we have of the theory and what we must do to really test it in a severe way.

Heavy leptons

At the 1976 Tbilisi conference the existence of a heavy lepton (τ) was controversial. In the two years since then its existence has been established beyond any doubt by a series of experiments at Stanford (SPEAR) and Hamburg (DORIS). These have made precise measurements of its properties; e.g. its mass is known to be within 0.5% of 1785 MeV (almost the mass of the deuteron!).

All the data suggest that the τ decays by weak interactions producing a (massless?) neutrino and that parity is violated in the process. It indeed appears that just as the electron and a neutrino (ν_e) are brothers in weak interactions (e.g. the radioactive β -decay of the neutron into proton, electron and ν_e), and that Nature repeated itself with a second pair (muon and its neutrino ν_μ), so now we have clear evidence that there is a third generation of leptons, the τ and its neutrino.

Each of these generations violates parity in weak interactions in the same way. It seems that each generation is identical but for two things: the τ is much heavier than the muon (100

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MeV) which is in turn much heavier than the electron (0.51 MeV); just as the muon does not transmute directly into an electron (*Nature* **266**, 679; 1977) so the τ does not directly turn into electron or muon.

The data on the τ decay patterns seem to be consistent with the above properties of the τ : decays which should not be seen indeed appear not to be there and decays which should be seen are found. In particular a problem with $\tau \rightarrow \pi + \nu$ (*News and Views* **271**, 407; 1978) has now gone away: the decay is seen at the expected rate.

Heavy quarks

Having at last established the existence of three generations of elementary leptons (*News and Views* **271**, 407; 1978) attention has turned to the questions of whether there is indeed a third generation of elementary quarks.

In the summer of 1977, the Y (up-silon) particle, 9.4 GeV in mass, was discovered in muon pairs produced by proton-proton collisions at Fermilab (*News and Views* **271**, 407; 1978). At the conference it was reported that the Y had now been seen by an independent Fermilab group and also by three groups working at the CERN Intersecting Storage Rings in Geneva.

The real excitement was provided by the groups working at the electron-positron colliding beam machine (DORIS) at Hamburg. It was reported a month ago (*News and Views* **273**, 705; 1978) that the Y had been directly produced in electron-positron annihilation, with properties suggesting that it was a bound state of a fifth flavour of quark (bottom) and its antiquark. Just a few days before the conference, the DORIS groups managed to produce a heavier version of this state (Y') with a mass of 10.01 GeV. Measurement of its coupling strength to photons and comparison with the group's data on the lighter Y were discussed and seem to indeed confirm that these are bound states of a bottom quark.

This precise mass determination was communicated to the group at Fermi Lab who had earlier found the Y in their proton experiment. They do not have the precision of DORIS but their data had shown an enhancement smeared out over 9 to about 10.5 GeV in mass which suggested that two or possibly even three Y -like particles were present. Now that the masses of Y and Y' were known so precisely they were able to look again at their data and found that it indeed requires the existence of a third Y (Y'') with predicted mass of 10.38 GeV. This discovery was telephoned to Tokyo with the recommendation that scientists at DORIS do a high precision search "between 10.379 and 10.381 GeV!".

This may turn out to be one of the first discoveries to be made at PETRA, the new higher energy electron-positron machine opening in Hamburg this month. What is interesting here is that only two Y states exist below charm production threshold and it had been predicted that three Y states should exist below bottom production threshold. This verification created much excitement for the PETRA programme since if the top quark exists (completing the third quark generation) then it will be much heavier still with the result that several θ states—bound states of top quark and antiquark—should exist below top production threshold. This gives the prospect of detailed investigation of quark dynamics by studying transitions among these levels of "toponium" analogous to transitions among levels of positronium and hydrogen in atomic physics.

The Hamburg group has studied the decay products of the Y . They have a different angular distribution than the particles produced in the electron-positron annihilation at energies just away from the Y resonance. This is precisely as predicted by QCD. However, it is not yet possible to be entirely sure that the effect is due to QCD as against random filling up of available phase space. This will become clear when the θ ("toponium") is found since at extreme energies the QCD theory predicts angular distributions quite unlike random phase space.

Future prospects

An experiment from Fermilab was reported that shows that particles containing b quarks are not stable, which implies that b quarks decay weakly. Decays of quarks in one generation producing quarks in another generation are familiar (*News and Views* **266**, 301; 1977) and are accommodated by the Cabibbo mixing theory. The decay of the b quark implies a new Cabibbo-like mixing involving the third generation with first or second generations. Weinberg stressed that (barring an accident) this necessarily leads to CP violation and hence one of the great puzzles of physics may be about to be solved. Indeed, such a theory of CP violation was postulated by Kobayashi and Maskawa in Japan as long ago as 1972. An important experimental programme will be to study the properties of b decays to see if they are quantitatively in accord with the magnitude of the CP violation which is known to exist.

If these expectations are all verified, then will this be the end of physics? Weinberg highlighted some of the remaining profound questions. Can we unify the strong with the weak and electro-magnetic forces? (some ideas

here were reviewed by Salam). Can we understand the various dimensionless parameters that pervade our universe, like the fine structure constant α , ratios of lepton masses and quark masses; the magnitude of the Cabibbo quark mixing angles and the weak-electromagnetic (Weinberg) mixing angle. The conjecture was that these answers may come as a consequence of a successful grand unification of the natural forces.

Lurking in the background was gravity. Grand unification may take place at energies like 10^{16} GeV (the Planck mass) and the reason for there being distinct forces with different strengths in our current experience may be due to the present coolness of the universe. So how might we achieve this grand unification? Weinberg revealed his ideas—a blank transparency. □

Echinoderm biology

from David Nichols

Two international meetings devoted entirely to echinoderm biology have taken place this year: in March, the Third International Echinoderm Conference was held at the Australian Museum, Sydney, and in July a meeting was organised principally for European workers at Bedford College, London.

At both meetings this year, the proportion of papers offered on the various groups of echinoderms was remarkably similar: notably, about a quarter at each were devoted to starfishes, and a tenth to palaeontological aspects. A major difference in the coverage was that at Sydney about a quarter of the papers were taxonomic, reflecting perhaps the present concentration on this type of work in the Australasian area, while in London only a single paper (by an Australian) was taxonomic.

It would have been all too easy for the Sydney meeting to have been dominated by papers on the coral-devouring Crown-of-Thorns Starfish, but the convener, F. W. E. Rowe (Australian Museum), wisely rationed this subject to three papers. One of these referred to the possible cyclic occurrence of population explosions in this starfish. Elderly fishermen have said that they recall abnormally large numbers of *Acanthaster* being present on the Great Barrier Reef at about the turn of the century, and E. Frankel (University of Sydney) has examined cores taken from the reef talus in several areas to see if he can identify abnormally high concentrations of the disarticulated skeletal ossicles of this starfish among

the broken coral and other material through the cores. He has assumed that the relative occurrence of these ossicles at the various levels in the cores might reflect the relative abundance of the starfish at the time the debris was being abraded from the reef. So far, Frankel has gone back 900 years, and, while not yet recording evidence of a plague 70 or 80 years ago (at the turn of the century), he has recorded higher-than-normal incidences of remains of *Acanthaster* at 110, 185 and 450 years BP.

The conveners of the London conference accepted two papers on *Acanthaster*. First, R. G. F. Ormond (University of York) described a computer model for the population strategy of the starfish, based on data from studies by the Cambridge Coral Starfish Research Group in the Red Sea. The model showed, for instance, that a rise in ambient temperature of a mere 2 °C could produce a population explosion from the 'background' level of 10 to 25 animals per kilometre of reef front to plague proportions of 130 or so. This suggests that *Acanthaster* may lie somewhere near the centre of the r-K strategy spectrum (in MacArthur and Wilson's terminology), and it may vacillate between low and high population levels, as suggested by the periodic plagues shown by Frankel's data.

The second paper on this animal was by J. Vasserot (Station Biologique, Roscoff, France) who suggested that a useful method of control in times of plague might be the use of hypersalinity, which he has found effective against the large European starfish, *Marthasterias*. The method consists of spreading common salt crystals over the aboral surface, and the treatment, which is non-polluting, results in death in a few days. In discussion after this paper, a contributor remarked that this method is already in use by Dutch shellfishermen, who transfer growing mussels from mussel-beds to the holds of their ships, subject them to high salinity, then return them to the beds. This treatment is apparently effective in killing starfish predators adhering to or hiding among the mussels, but does not kill the mussels themselves.

At one time a possible biological control of the Crown-of-Thorns was suggested, which involved seeding the infested reefs with a shrimp, *Hymenocera*, which was known to attack starfishes by piercing the body wall and extracting coelomic fluid. The method was not successful, but it focused attention on the relationship between shrimps and echinoderms. It later began to emerge that such associations are more commonly symbiotic or commensal than predatory. At the Sydney meeting, A. J. Bruce (Director, Heron Island Research Laboratory, Queensland) gave a survey of such associations

in the Indo-West Pacific region, and estimated that about 11% of known shrimps in these waters form some sort of association, mainly with starfishes, sea-urchins and feather-stars. Many use the host merely as a retreat, and emerge on to the substrate to feed; sometimes there may be as many as 50 shrimps on one host; colour patterns in a single species differ according to the host and are often highly cryptic; diurnal colour change has been observed; and some shrimps shave a sea-urchin of some of its spines to make a 'clearing' in which to reside. Little is yet known of these associations, clearly a rich field for future work.

Still with interspecific associations, a highly unexpected spin-off came from some conventional studies of reproductive cycles in the common Australian cushion-star *Patriella exigua* by D. T. Anderson and C. Lawson-Kerr (University of Sydney), this time between the starfish and a terrestrial caddis-fly. *Philaniscus* is a caddis that is known to inhabit the littoral zone in Australia, but until now its intermediate host has been a mystery. During routine sectioning of the gonads of the starfish, these workers observed some egg-strings that clearly did not belong to the starfish, and these were subsequently identified as belonging to the caddis-fly. The female fly was later seen to settle on the dorsal surface of the starfish at low tide and penetrate the dorsal integument in the region of the gonads with a long ovipositor. As yet, the emergence of the young flies has not been observed. This must be one of the very few examples of echinoderms used as intermediate host to a terrestrial organism.

At both meetings there were papers on one of the most controversial aspects of the present-day study of echinoderms, namely, the interpretation of the structure and relationships of a group of fossils variously called carroids, homalozoans or heterosteles. Most echinoderm biologists interpret the unusual fossils in purely echinoderm terms, with a flexible plated body pierced by the usual echinoderm orifices and with various appendages protruding from the body for fixation or feeding. However, in the late 1960s R. P. S. Jefferies (British Museum (Natural History), London) produced a new interpretation of them in mainly chordate terms, referring to them as calcichordates on account of their calcite skeletons. At the London meeting, Jefferies showed how he views both echinoderms and chordates as descended from an ancestor that had settled on the right side of the body (the dextiothetic condition). The calcichordates were seen as the primitive chordate descendants and included forms regarded as primitive cephalochordates, tunicates and vertebrates.

He proposed the term Dextiothetica for the echinoderms and chordates together. The most primitive calcichordates were remarkable in having gill-slits on only the left side of the head, and he pointed out that in the development of the living primitive chordate *Amphioxus* there is a comparable stage with left gill slits only. Jefferies explained this as a straightforward case of Haeckelian recapitulation.

This view has not yet won general acceptance, and at the Sydney meeting G. Philip (University of Sydney) gave a short critique of what he saw as the main points of conflict between the two principal views of these animals. For instance, the 'chordate' school, principally Jefferies, sees the projecting structure of cornutes such as *Cothurnocystis* as a propulsive organ, complete with ossicles ventrally with large muscles above them which were roofed over by immoveable plates; the same structure is seen by those who view the animals as echinoderms as a typical feeding ambulacrum, complete with groove for radial nerve and water vessel, cups for the origin of tube-feet and openable coverplates for protection, very much like an arm of a modern sea-lily or brittle-star. There is clearly a wide divergence of view on the interpretation of these animals, and the hope was expressed in discussion after Philip's paper that the next International Conference should devote a session to this one topic. Much rests on the presumed occurrence of certain types of soft tissue adjacent to the fossilised hard parts that now remain, and perhaps research now underway in Exeter on the nature of the calcite that underlies various kinds of soft tissue will be able to offer additional evidence on whether, for instance, large propulsive muscles really did lie along the projecting stalk of carroids, or whether the crystalline structure of the skeleton in that region is more consistent with other interpretations.

There may now be a break-through in the study of the echinoderm nervous system. Hitherto, workers have been hampered by the difficulty in recording either intra or extra-cellular potentials from individual nerves, because of their close packing and mostly small size. Now, however, it looks as though recording is possible, at least in those echinoderms which have recently been shown to possess giant fibres. An American worker, P. H. Brehm, has succeeded in recording extracellularly from a single nerve axon, using refined suction electrodes on the giant fibres in the radial nerve cord of a brittle-star. J. L. S. Cobb (Gatty Marine Labora-

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tory, St. Andrews) reported to the London meeting that he has repeated Brehm's technique, and has recorded the potential along over 70 cm of a single brittle-star nerve upon photic stimulation. The way may now be open for much further progress in the study of the neurological basis of observed echinoderm behaviour which has until now proved so difficult.

Research effort in echinoderm biology is increasing. An *Echinoderm Newsletter* produced by D. L. Pawson

and M. E. Downey (US National Museum, Smithsonian Institution, Washington) is mailed to over 700 workers, and the three International Conferences have generated a surprisingly large response. At the two meetings this year there was talk of establishing a learned society devoted entirely to echinoderm biology, and of seeking support for a journal. Maybe this reflects a growing awareness that many marine habitats are dominated by this group of macroinvertebrates. □

Recombination in semiconductors

from P. T. Landsberg

RECOMBINATION processes in semiconductors have become increasingly important since the invention of the transistor. In semiconductor devices switching and storage times are governed by the recombination lifetimes of excess carriers, as is the photo-voltage generated in solar cells, and in light-emitting diodes it is the ratio of radiative to total life times that plays a crucial role. Fundamental work in the field of recombination was the subject of a meeting on Recombination in Semiconductors which took place in Southampton at the end of August. Sir Neville Mott gave the opening address and his support and interest was widely appreciated by the participants.

Many of the semiconductor mechanisms—certainly radiative and Auger recombination—are of interest in plasma physics. This includes the cascading of a non-equilibrium electron down a ladder of states with the emission of a boson at each step. In plasmas and gaseous nebulae for example the boson is a photon and we then have a radiative cascade. Another recent development in this field is the hybrid process of a phonon cascade and multi-phonon process for the last step, when the electron reaches the ground state of a deep centre from its first excited state (N. F. Mott, Cambridge).

What other bosons are there? Should we not also expect in due course to be working on a plasmon-cascade, as narrow gap semiconductors become the subject of more accurate experiments? Although this process does not appear to have been considered before, electron-hole recombination can be greatly increased if a narrow energy gap can be tuned to the plasmon or optical phonon energy. Photoconductivity measurements on semimetallic $\text{Hg}_{1-x}\text{Cd}_x\text{Te}$ alloys reported by Dornhaus and Nimtz (Cologne) have

now confirmed this view. Of course for far infra-red devices it is desirable for good detectivities to find ways of lengthening life times rather than shortening them; coherent plasmon emission is, however, brought closer.

The analogy between semiconductors and plasmas is driven home by low temperature photoexcitation. The electron-hole pairs may then be so tightly packed that there is not enough space for exciton orbits of 100 Å or so, and one then has an electron-hole plasma or liquid. Just as there are droplets formed in the transition between water and water vapour at high humidity, so one has in this case the possibility of drops of electron-hole liquid in an exciton gas. The drops can be made to coalesce by the application of strain fields and so can reach radii from 50 μm to 7000 μm. In a dynamic equilibrium excitons condense on the drops and split up into electrons and holes so as to replenish the pairs lost by recombination within the drops. Studies of these effects were reported by Kelso and Furneaux (Univ. of Calif., Berkeley) who suggested the importance of a constant (i.e. independent of the pair density) term in the drop recombination rate. The experiments can be done for example by studying the decay of luminescence after laser cut-off. While Auger recombination dominates in unstressed drops in Ge, it may be that impurity recombination gives rise to the constant term and dominates in the stressed case. The radiative recombination from the droplets may be expected to be about 8 Mev wide by considering the separation between the quasi-Fermi levels.

Using appropriate reduced variables yields phase diagrams for electron-hole drops which are remarkably similar to those found many years ago for a number of gases. For lower excitation one

tends to have an exciton gas without drops, and no drops form above a critical temperature. Other highly interesting recombination effects in the e-h plasma include the possibility of populating the direct minimum in Ge by a band-band Auger effect (Klingenstein and Schweitzer, Stuttgart Univ.).

The weaker concentration dependence of the Auger effect in degenerate ($\sim np$) compared with non-degenerate ($\sim n^2p, np^2$) materials is relevant when setting up the steady state equations for droplets. A stronger dependence even in the degenerate material can again be obtained if k-conservation is neglected or because of higher order effects ($n^{7/3}p$) as has been shown by Haug (*Solid-State Commun.* **22**, 537; 1977).

When we come to bound excitons, it was found some years ago that radiative decay dominates for direct gaps. In an interesting report it was suggested that for small direct gaps the Auger recombination dominates over the radiative mechanism. As the gap is made smaller the Auger effect comes more easily and the radiative transition is less easy. This can be studied in $\text{Hg}_{1-x}\text{Cd}_x\text{Te}$ whose energy gap E_g can be changed by varying x . The two mechanisms are equally important for $E_g \sim 0.5$ eV in this case (Osborn *et al.*, Caltech, Pasadena). The temperature dependence of the Auger effect gave $\sigma_A \propto n_{ex}^{-8/3}$ instead of n_{ex}^{-2} , where n_{ex} is the extrinsic carrier concentration (Gerhardt, Berlin and Dornhaus & Nimtz, Cologne). The luminescent decay kinetics for excitons bound to isoelectronic traps in the GaP (the light-emitting diode material) was discussed by Sternheim and Cohen (Haifa).

Multiphonon processes are another field of current interest. The configuration coordinate diagram for ground and excited states of a centre show that (for broadened vibrational energies) one can have at one and the same energy a low-phonon excited state and a many-phonon ground state. The multiphonon transition can be visualised in this way. Indeed the energy given up in this type of recombination (and also in some other mechanisms) can pass to a defect and enable it to diffuse or dissociate (Kimerling, Bell Labs.). One application of this idea (Hutchinson, Birmingham) is that a dislocation emits vacancies into the surrounding lattice as the recombination energy is dissipated, thus increasing trapping defects and causing degradation in devices such as lasers. If this were a

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key degradation process, one would try to use dislocation-free material in these semiconductor devices. In solar cells the lattice perfection is of importance (Kazmerski, Solar Energy Research Institute, Golden), as is recombination as a limit on efficiency (Klimpke and Landsberg, Southampton).

Just as it is desirable to design experiments which make the energetic Auger electron visible in some sense when the Auger effect is believed to be important, so also in phonon transitions it is useful to have as direct a way as possible to find evidence for the emitted phonons. This was recently shown to be possible by experiments on GaAs using a superconducting bolometer consisting of a granular Al film (Narayanamurti *et al.*, *Phys. Rev. Lett.*, **40**, 63; 1978). They identified phonon emission and explained the fraction of longitudinal and transverse phonons in terms of selection rules. This work has now revealed an anisotropy in phonon generation when observations are made along different crystallographic directions, using n-GaAs epitaxial layers and also p-n junctions. There is a pleasing correlation with light output of dislocated p-n junctions suggesting that this process is an important mode of non-radiative recombination.

It is known that in GaP grown by liquid phase epitaxy dislocations limit the lifetime and hence the radiative output of green light-emitting diodes. Dimitradis *et al.* (UMIST, Manchester) showed how the lifetime increases in GaP as one leaves the neighbourhood of a single dislocation line, and how it approaches the bulk dislocation-free value at the distance of one diffusion length.

The radiative transitions between Landau levels in n-InSb observed in 1972 have now been studied further to find that the lifetime of excess carriers in the first Landau level is inversely proportional to the concentration in this level (Müller & Gornick, Vienna and Bridges & Chang, Bell Labs). Short times of $\sim 10^{-10}$ s are found for concentrations of the order of 10^{13} cm $^{-3}$. A new but expected effect which was reported by Street *et al.* (Xerox, Palo Alto) is the magnetic field enhancement of luminescence in amorphous silicon. It is related to the anomalous photoconductivity and one wonders how general this effect is, since a magnetic field can cause an increase in lifetime by a factor of order 20 at 4 K in Hg $_{1-x}$ Cd $_x$ Te.

Finally, a new technique of recombination centre photocurrent analysis suitable for inhomogeneous distributions of recombination centres was proposed by Lang & Henry of Bell Laboratories. □

Has Atlantis disappeared again?

from D. H. Tarling

THE popular imagination has been captured by the idea that the destruction of Atlantis, as outlined by Plato from Egyptian sources, was in fact a record of the eruption of the Thera volcano in the Aegean some 3,500 years ago—which destroyed the Minoan civilisation not only on the volcano itself, but also on Crete and the surrounding islands. (S. Marinatos *Antiquity*, **13**, 425; 1939, J. C. Luce *The End of Atlantis* Thames & Hudson, 1969). At the same time, scientists concerned with the area who had previously promoted this image seem to be revising their views and are now proposing only tenuous links between the eruption and the decline of the Minoan thalocracy. The problem is that there seems to be a distinct interval between the two events of the order of 50 years. If such a gap really exists, it seems unlikely that the civilisation was ended either by tsunami destroying their fleets or by volcanic ash-fall destroying their crops. This interval is of critical interest to archaeologists and historians, but the succession of events during the largest volcanic eruption in history—at least four times as powerful as the 1883 Krakatoa eruption—is also of interest to a much wider audience. To discuss these matters of common interest archaeologists and Earth scientists gathered for the 2nd International Thera Congress, sponsored by Peter Nomikos, and held aboard a ship cruising the relevant areas of the Aegean.

The geophysical-geochemical background to the Thera volcanic activity was, naturally, based on plate tectonic models in which an East Mediterranean plate is descending to some 110–140 km beneath the Aegean, from which volcanic magmas are currently being derived (H. Berckhemer, Frankfurt; D. Kiskyras, Athens; B. C. Papazachos & P. D. Comninakis, Thessalonika). Geochemical evidence of major, trace and rare earth distribution were in remarkably good agreement for a model in which water released from the descending plate caused partial melting in the mantle wedge overlying it (I. A. Nicholls, Monash; H. Puchelt, Karlsruhe; R. S. Taylor, Canberra) and the magmas are therefore unrelated to descending slab, except indirectly, and are also virtually free of contamination from the thin, overlying Aegean crust. The Aegean, in fact, seems to have a thin crust that is currently in tension (N. C. Fleming, Godalming)—a surprising observation

when it is considered that most of the surrounding areas are under strong compressive stress. This situation is probably related to convective circulation beneath this marginal basin, although it is important to distinguish major differences between this area and the Pacific margin. Around the Western Pacific, earthquake activity proceeds to much greater depths than in the Aegean and oceanic crust is being subducted, whereas the Eastern Mediterranean crust is generally considered to be exceptionally thick oceanic or thin continental crust.

However, as earthquake activity in the Aegean has only been recorded in detail for something like the past 50 years and only with adequate precision for the past 10 years or so, comparison with the Pacific margin is still needed to evaluate possible relationships between earthquake and volcanic activity. C. Blot (Solles-Pont) made analogies with Guatemala, in particular, to predict that major earthquakes about 100 km beneath Thera would be followed some 1½ to 2 years later by either an eruption or major shallow earthquake on Thera and that this would be followed, some 3 years later, by strong shallow earthquakes in the area of Crete. Unfortunately the precise relationship between earthquake and volcanic activity is still to be firmly established, and many earthquakes seem to be unrelated, in any direct fashion, to magma migrations. It is difficult therefore to apply such models to explain the apparent time-gap between the archaeological evidence for earthquake activity and subsequent volcanic activity and then further possible earthquake activity.

Surprising unanimity was also expressed by volcanologists attending the Congress. There was, for example, agreement that the volcanic deposits were related to only one cycle of volcanic activity and that this activity, by analogy with Krakatoa 1883, probably took place during a single day (C. J. & D. Vitaliano, Indiana; H. Pichler, Tübingen; W. L. Friedrich, Aarhus, R. W. Decker, Dartmouth). This contrasts with previous interpretations of some ash-falls as being related to up to three distinct eruptions, with possible intervals of a decade or so between each fall. This unanimity reflects increasing understanding of volcanic processes and geochemical evidence for the uniformly increasing basicity of the tuffs as the pumice was generated (C. J. Vitaliano *et al.*, Indiana). It is now clear that the earth-

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quake that destroyed the Minoan city of Akrotiri, on Thera, occurred long enough before the eruption—to allow people to return and attempt some initial clearance (C. Doumas, Thera). The eruption itself clearly started with an ash-fall of ~1–2 m thick over the city and was preceded by a very fine pumice fall, only some 1–2 cm thick, which may have been sufficient warning for those clearing the site. The main pumice, however, seems to have been in the form of a base surge, followed by a chaotic ash-flow stage during which large volcanic bombs continued to be spouted out of the volcano. There were clearly no distinct *nuée ardente* deposits, although some pumice debris on wall frescoes has been used to determine the direction of flow of these glowing dust clouds (P. Hédervari, Budapest). One of the most puzzling observations was that preliminary palaeomagnetic data indicated that the walls of the Minoan city had not even been warmed by the volcanic debris and that both the ash-fall, base-surge and ash-flow deposits seemed to have been emplaced very close to room temperature (D. H. Tarling, New-castle), thereby indicating mud-flow origin (A. Bond & R. S. J. Sparks, *J. Geol. Soc.*, **132**, 1: 1976) at ambient temperatures. Whatever the origin, there was clear agreement that these deposits were all accumulated in a very short time so that the interval between the Thera eruption and the end of the Cretan thalocracy could not be interpreted in terms of a succession of eruptions spread over, for example, 50 years. There was also surprising agreement on the improbability of the pumice ash, that undoubtedly fell on eastern Crete (F. W. McCoy, Columbia), having any great effect on the harvest for that or subsequent years unless the pumice also included poisonous gases, such as fluorine (D. Vitaliano, Indiana). It was even suggested that the thin ash-fall, at most 5 cm thick, could have been beneficial.

The effects of tsunami (I. Yokoyama, Hokkaido), associated with either the earthquake or volcanic eruptions, received surprisingly little direct attention. In general, most earth scientists were mainly concerned with establishing that any tsunami would not have affected ships at sea and any effects would be restricted to the damage of ships at anchor and harbour installations. There is little evidence for harbours along northern Crete at that time (N. C. Fleming, Godalming)—an observation which received little comment yet must have important implications for the idea of a thalocracy governed from Crete. The only solution seems to be that the catastrophic series of events had such a psychological im-

pact that any religious-based administrative system would have collapsed. Nonetheless, such a disintegration would still be expected in late Minoan IA times and not up to 50 years later. The ^{14}C dating of both short-lived materials (grain, shrubs, burnt bone for example) and long-lived materials has raised more problems than it has solved (P. Betancourt, M. Biddle & E. Ralph, Philadelphia). Unlike most other sites, both types of material show an extraordinary range in values, with 70% of the ages for both types of material more than one standard deviation older than the expected 1500–1400 BC date—an age which is known from the good correlations between the precise Egyptian chronology and Minoan pottery styles on Crete. Although the Congress was only presented with the carbon dates, it seems that the only sensible interpretation is that the region of Akrotiri was completely abnormal for well before the earthquake which was to destroy it. It seems unlikely that the ^{14}C dates have been affected by later contamination as most of the materials were well sealed with the archaeological deposits, so the most likely, but still imponderable, explanation is that the vegetation in the area was accumulating varying degrees of old carbon in its structure whilst growing. The processes by which this occurred are unclear, as any volcanic gas emanations would rapidly mix with the atmosphere and clearly much more detailed study is required to establish how such a situation may arise as it is clear that the normal assumptions on which ^{14}C is based do not seem to be valid here and must raise doubts as to their validity in other situations, until the cause of the internal scatter in the Akrotiri materials can be satisfactorily explained.

The archaeological basis for a difference in age between the Thera eruption and the Minoan decline has changed little. In general, most archaeological and historic studies confirm that there was a real time-gap between the Late Minoan IA pottery motifs found in Thera and those extant in the Late Minoan IB period in Crete at the time of the takeover by the Mycenaeans (S. Hood, Oxford; A. Furumark, Uppsala). The evidence suggests that the takeover was not a deliberate invasion but that a Mycenaean exploratory fleet came across a Minoan Empire that was in complete disarray for unclear reasons.

To some extent, therefore, Earth scientists have provided a somewhat more uniform picture of the sequence of events during the Thera earthquake and eruption, but such models are still likely to be drastically modified if archaeological evidence can be ob-

tained for a revision of the relationship between several earthquake events during the period 1500–1400 BC and the volcanic eruption. It will be surprising, however, if such a major series of tectonic events should not be directly related to the otherwise puzzling, rapid decline of the Minoan civilisation at a similar time. □



A hundred years ago

AMONG the resolutions passed by the International Congress on Weights, Measures, and Coins, at Paris, was the following:—The Congress learns with pleasure the progress of the metric system; it deplores that England, Russia, and the United States have not yet entered into the same path; and it is of opinion that the Governments of those countries should be solicited to give effect as early as possible to an act of progress so eminently useful to science, commerce, and international relations." The British and American members had a separate meeting, and resolved to petition their respective Governments to appoint a mixed commission to consider adoption of the metric system by both countries, and to make all necessary recommendations for the proper legislation to secure the desired end.

I AM able to confirm the accounts given by Mr. Simson in your last number as to the probability of the hearing of insects. When travelling on the River Magdalena, New Granada, in 1861, the mode of which is by a long boat, arched over with bamboo, on which the sailors (bogás) passing from one extremity to the other, propel it with long poles, hugging the river bank, accompanied with wild cries and execrations, I observed on several occasions that these cries suddenly ceased, a dead silence following, and on inquiring the cause they pointed to nests high up in the trees, whispering the word *vispa* (wasp). As the bogás pursue their avocations in a state of semi-nudity, they have the greatest dread of these insects, fearing to speak aloud, as their only alternative if attacked by them is to plunge into the stream, where alligators abound. The wasp is long, slender, and black in colour.

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review article

The tau heavy lepton—a recently discovered elementary particle

Martin L. Perl*

This review recounts the history of the discovery of the tau heavy lepton. Evidence for the tau being a lepton is summarised and the known properties of the tau are discussed.

THERE is now very substantial evidence for the existence of a new elementary particle called the tau (τ). (The name τ is taken from the first letter of the Greek word $\tau\alpha\upsilon\rho\nu$. It signifies that the τ is the third charged lepton to be found, the first two being the electron and the muon.) Elementary particles are the simplest forms of matter and through studying them we hope to understand the fundamental nature of matter. As I will show in this article, the properties of the τ are analogous to the properties of the electron (e) and muon (μ). Therefore, the τ has been classified as a member of the lepton particle family (Table 1) even though it is much heavier than the e or μ , having a mass of almost $1,800\text{ MeV}/c^2$. The discovery of this new lepton has particular significance in the world of elementary particles because the lepton family has so few members, and because the τ is so relatively massive. One purpose of this article is to discuss that significance; the other purposes are to outline the evidence for the existence of the τ and to describe its properties as far as we know them.

The first evidence^{1,2} for the existence of τ leptons was found at the SPEAR electron-positron colliding beams facility of the Stanford Linear Accelerator Center (SLAC). The τ 's were produced through the electron-positron annihilation reaction

$$e^+ + e^- \rightarrow \tau^+ + \tau^- \tag{1}$$

a simple reaction whose theory is well understood. All subsequent studies of the τ at SPEAR and at DORIS, the electron-positron colliding beams facility at the Deutsches Elektronen-Synchrotron (DESY), have used reaction (1). (See refs 3–6 for reviews on τ .) Therefore, in this article, I shall limit my discussion of the production of τ 's or other heavy leptons to this reaction.

The unique position of the leptons in elementary particle physics is illustrated by Table 2 which summarises the differences between the lepton and hadron families. The hadrons are composite particles made up of constituents we call quarks; the hadrons interact through the strong interactions which at high energies can lead to very complicated reactions in which many hadrons are produced; and the hadrons occur in more than 100 different types. (See refs 7, 8 for reviews on quarks.) In stark contrast: the leptons are simple, nondivisible particles; they do not partake in the strong interactions; even at high energies the reactions in which the leptons directly play a part only produce a few particles (that is, the lepton vertex in the Feynman diagram of the reaction produces only a few particles); and as I have already noted, there are only a few types of leptons. Therefore, the leptons, in contrast to the hadrons, are truly

elementary particles; and in studying the leptons we are studying directly the simplest forms of matter which we have yet discovered. The quarks may also be truly elementary particles; however, we do not know if they can be isolated in a free state; and at present, we can only study them very indirectly. Until the discovery of the τ one might have thought that the simplicity and truly elementary nature of the leptons were connected with their small mass. The e , the μ and their neutrinos have less mass than the lightest hadron—the π^0 with a mass of $135\text{ MeV}/c^2$. (The name lepton means 'light one' in Greek.) However, the τ is heavier than many hadrons and its existence once and for all separates the concept of a particle's elementarity from the size of a particle's mass. Indeed, we emphasise this by referring to the τ by the oxymoron 'heavy lepton'.

Lepton conservation and the sequential heavy lepton model

Our search for heavy leptons which led to the discovery of the τ was based on the sequential heavy lepton model⁹. We could have used other models^{10,11} in our search; however, the elegance, symmetry and simplicity of the sequential model appealed to us. To our surprise, the properties of the τ are consistent with its being a sequential heavy lepton. The sequential model is based on extending the concept of lepton conservation to heavy leptons. Lepton conservation is the explanation of why reactions such as

$$\mu^- \not\rightarrow e^- + \gamma \tag{2}$$

$$\text{or} \quad e^+ + e^- \not\rightarrow \mu^+ + e^- \tag{3}$$

do not occur. Taking reaction (2) as the simplest example; lepton conservation asserts: (1) that the e and the μ each possess a unique property not possessed by the other particle, (2) that those properties cannot be destroyed in a reaction, and hence (3) that a μ cannot change into an e . This concept is codified in a

Table 1 The lepton particle family

Name	Electron	Muon	Tau
Charged lepton	$e^\pm$	$\mu^\pm$	$\tau^\pm$
Charged lepton mass (MeV/c^2)	0.51	105.7	$1,782^{+2}_{-7}$
Charged lepton lifetime (s)	Stable	2.20×10^{-6}	$<3.5 \times 10^{-12}$
Associated neutrino	$\nu_e, \bar{\nu}_e$	$\nu_\mu, \bar{\nu}_\mu$	$\nu_\tau, \bar{\nu}_\tau$
Associated neutrino mass	$<60\text{ eV}/c^2$ (may be 0)	$<0.57\text{ MeV}/c^2$ (may be 0)	$<250\text{ MeV}/c^2$ (may be 0)

* Stanford Linear Accelerator Center, Stanford University, Stanford, California 94305.

Table 2 Comparison of leptons and hadrons		
Family	Lepton	Hadron
Structure	Simple particle, no structure or constituents found by experiments	Composite particle constituents are quarks
Interacts through strong interactions	No	Yes
Interacts through electromagnetic interaction	Only if charged	Yes, in all cases
Interacts through weak interactions	Yes	Yes
Interacts through gravitational interaction	Yes	Yes
No. of types found so far	6 if ν_τ is unique	>100

more mathematical form in Table 3 where the e^- and its associated neutrino ν_e are assigned the 'electron lepton number' $\eta_e = +1$, and so on. Lepton conservation then states that in any reaction the separate sums of η_e and η_μ on one side of a reaction must equal their respective sums on the other side. For example: reaction (2) does not occur because on the left side $\eta_e = 0$ and $\eta_\mu = 0$ while on the right side $\eta_e = +1$ and $\eta_\mu = -1$. In contrast to reaction (3), the reaction

$$e^+ + e^- = \mu^+ + \mu^- \tag{4}$$

can occur because η_e and η_μ are zero on both sides. Indeed, reaction (4) is a well known and well studied reaction.

An important consequence of lepton conservation is that decay of the μ to an e occurs not through the simple reaction in equation (1) but through the complicated reactions

$$\mu^- \rightarrow \nu_\mu + e^- + \bar{\nu}_e \tag{5a}$$

$$\mu^+ \rightarrow \bar{\nu}_\mu + e^+ + \nu_e \tag{5b}$$

It is important to note that the decays in equation (5) take place through the weak interactions. The μ cannot decay through the strong interactions because it is not affected by the strong

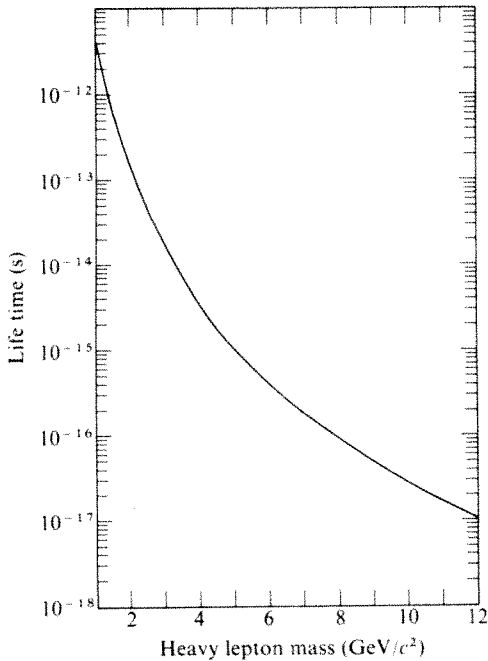


Fig. 1 Theoretical prediction for the lifetime of a sequential heavy lepton with V-A coupling and conventional weak interactions.

interactions; and the μ cannot decay through the electromagnetic interaction because the electromagnetic decay channel, reaction (2), violates lepton conservation. Lepton conservation also dictates how hadrons can decay to leptons. For example, the decays

$$\pi^- \rightarrow \mu^- + \bar{\nu}_\mu \tag{6a}$$

$$\pi^- \rightarrow e^- + \bar{\nu}_e \tag{6b}$$

can and do occur.

Before returning to the sequential heavy lepton model, I should remark that we have no deep understanding of lepton conservation; that is, we do not know how or in what way a μ differs from an e . As an experimenter, I regard lepton conservation simply as an empirical rule whose underlying significance we may one day understand through further experimentation with leptons.

The sequential heavy lepton model⁹ simply assumes that there is a mass sequence of charged leptons and associated neutrinos:

Charged lepton	Associated neutrino
$e^\pm$	$\nu_e, \bar{\nu}_e$
$\mu^\pm$	$\nu_\mu, \bar{\nu}_\mu$
$\tau^\pm$	$\nu_\tau, \bar{\nu}_\tau$
$\vdots$	$\vdots$

It further assumes that each type of lepton has a unique lepton number which is separately conserved in every reaction. Using this idea and assuming that the mass of the τ (m_τ) is sufficiently large, the τ should decay through the reactions

$$\tau^- \rightarrow \nu_\tau + e^- + \bar{\nu}_e \tag{7a}$$

$$\tau^- \rightarrow \nu_\tau + \mu^- + \bar{\nu}_\mu \tag{7b}$$

Table 3 Values of lepton numbers for various particles			
Particle	Electron lepton number, η_e	Muon lepton number, η_μ	Tau lepton number, η_τ
e^-, ν_e	+1	0	0
$e^+, \bar{\nu}_e$	-1	0	0
μ^-, ν_μ	0	+1	0
$\mu^+, \bar{\nu}_\mu$	0	-1	0
τ^-, ν_τ	0	0	+1
$\tau^+, \bar{\nu}_\tau$	0	0	-1
All hadrons	0	0	0

This table assumes the τ is a sequential heavy lepton with its own lepton number η_τ .

These decay channels are analogous to the μ decay channel in reaction (5a): they take place through the weak interactions and they obey lepton conservation. (In the remainder of this article, I shall only write decay reactions for the τ^- , as the τ^+ decay can simply be obtained by reversing the signs of all the charged particles and interchanging neutrinos and antineutrinos.) An exciting consequence of this model is that a sufficiently massive heavy lepton ought to decay to hadrons—just the reverse of reaction (6) in which a hadron decays to leptons. For example, decays such as

$$\tau^- \rightarrow \nu_\tau + \pi^-$$

$$\tau^- \rightarrow \nu_\tau + \pi^- + \pi^0 \tag{8}$$

and

$$\tau^- \rightarrow \nu_\tau + \pi^- + \pi^+ + \pi^-$$

should occur. These decays would occur through the weak interactions.

The lifetime of a sequential heavy lepton decreases as its mass increases because as the mass of a particle increases it becomes more unstable, and because more decay channels, such as those in equation (8), are available. If one assumes that the sequential

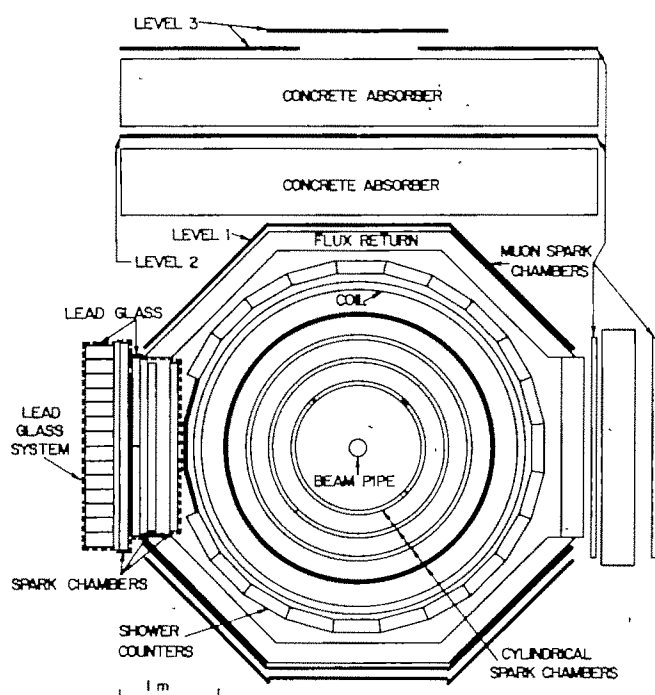


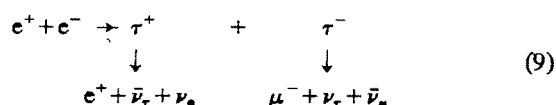
Fig. 2 A cross-sectional view of the SLAC-LBL magnetic detector. The e^- and e^+ beams move along an axis (perpendicular to the paper) inside the beam pipe. The concrete absorber was an addition to the detector used to detect μ -hadron events from τ decays.

heavy lepton has the same type and strength of weak interactions as the μ and e , then its lifetime can be calculated¹¹⁻¹³ (Fig. 1). When we began our search, we already knew that there were no sequential heavy leptons with masses less than 1,000 MeV/ c^2 . This lower limit had been set by experiments^{14,15} carried out at the ADONE electron-positron colliding beams facility at Frascati, Italy. Therefore, as shown in Fig. 1, the heavy leptons we were seeking would have lifetimes so short that they would decay before reaching our detection apparatus; hence they would have to be detected through their decay products. How this was done is the subject of the next section.

Evidence for the τ particle

$e\mu$ Events. We used the SLAC-LBL magnetic detector at SPEAR for our search (Fig. 2). This detector gathered information on electron-positron annihilation events in which at least two charged particles appeared in the apparatus. We knew that the vast majority of these events had nothing to do with heavy leptons. However, we searched through the events for the events in which only two particles were observed: one an electron and the other a muon, the two particles having opposite electric charge.

These $e\mu$ events could come from the reaction and decay sequence



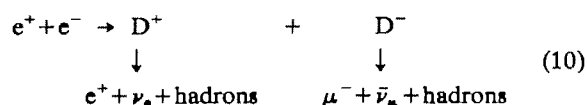
as neutrinos pass unobserved through the detector (Fig. 3). This is a strong signature for heavy lepton production because lepton conservation forbids the production of only an e and μ in the final state; and there are very few reactions in which only an e , a μ and neutrinos are produced. Furthermore, reaction (9) is distinctive because there is on the average a large amount of energy carried off by the neutrinos and hence unobserved.

In the winter of 1974-75 we began to find^{1,2} $e\mu$ events when the total energy, E_{total} , of the e^+ and e^- beams at SPEAR was above $\sim 4,000$ MeV. Because in the reaction $e^+ + e^- \rightarrow \tau^+ + \tau^-$ two τ 's must be produced, this meant that the mass of the τ had to be less than 2,000 MeV/ c^2 . We were also able to place a

lower limit of about 1,600 MeV/ c^2 on m_τ by studying the momentum distribution of the e and μ in reaction (9). By the summer of 1976, we were able to show¹⁶ that all the properties of the 105 $e\mu$ events we had by then accumulated were consistent with these events being from heavy lepton production.

Figure 4 illustrates one of the consistency tests. The momentum spectrum of the e or the μ produced in reaction (9) through the three-body decays of the τ 's, reaction (7), is described by the solid curve in Fig. 4a. If the τ decayed differently, say by a two-body decay $\tau \rightarrow e + \nu$ or $\tau \rightarrow \mu + \nu$, then the dashed curve would result. Figure 4b shows our measurement¹⁷ of the e and μ spectrum. We could not detect the slow e 's or μ 's required for the low momentum, ascending part of the solid curve in Fig. 4a; however, our measurement was clearly consistent with the high momentum part of that curve. And our measurement is clearly inconsistent with the two-body decay of the τ . A later but more complete measurement of the e momentum spectrum from $e\mu$ events is shown in Fig. 4c. This was obtained by Burmeister *et al.*¹⁸ using the PLUTO detector at DESY.

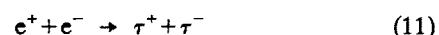
Of course, the existence of a heavy lepton is not demonstrated simply by showing that $e\mu$ events have properties consistent with those of a lepton. One must also show that there is no other source of the $e\mu$ events. One alternative source which was often suggested was the production and decay of a pair of charmed mesons.



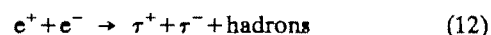
If the final hadrons were not detected, then reaction (10) might resemble reaction (9). My colleague, Gary J. Feldman, was able to show¹⁶ that the majority of our $e\mu$ events did not have undetected hadrons accompanying them.

A very clear proof that $e\mu$ events did have accompanying hadrons, detected or not detected, was produced by the group using the PLUTO detector. They showed¹⁸ that in a data sample which contained 23 $e\mu$ events there were no events of the type $e\mu + \text{hadrons}$.

There was another important result of demonstrating that $e\mu$ events did not have accompanying hadrons. It showed that the reaction



and not the reaction



was the source of $e\mu$ events. We do not expect reaction (12) to

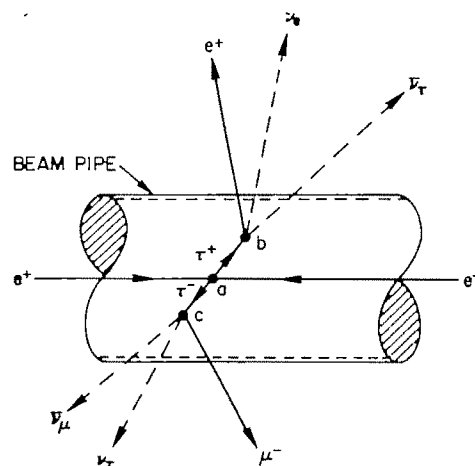
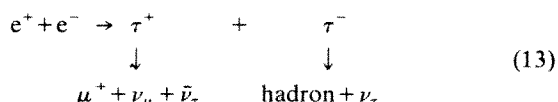


Fig. 3 A schematic diagram of how $e\mu$ events are produced. The τ 's are produced at point (a) where the e^+ and e^- beams collide. They decay close to point (a) at points (b) and (c) while still inside the beam pipe (see Fig. 2). Therefore only the decay products reach the detector. However, the neutrinos, indicated by dashed lines, cannot be observed in the detector, only the e and μ are observed.

be a source of heavy leptons, and the absence of reaction (12) was an additional proof that the τ was a lepton.

μ -Hadron events. If we accepted the $e\mu$ events as coming from the production and decay of a pair of τ heavy leptons, then the sequential heavy lepton model and the mass of the τ being greater than $1,600 \text{ MeV}/c^2$ would require the existence of other rather strange types of events. Thus the reaction and decay sequence



should occur, where the $\tau^- \rightarrow \text{hadrons} + \nu_\tau$ decay is one of the types in equation (8). These events are called μ -hadron events. We were particularly interested in the μ -one-charged-hadron events in which there was just one charged hadron, because such events were unlikely to come from other sources such as charmed meson production. In 1976 a small sample of μ -one-charged-hadron events was found at SPEAR by Cavilla-Sforza *et al.*¹⁶. Later in 1976, after having added an improved muon detection system to the SLAC-LBL magnetic detector (Fig. 2), we were able to report the finding of a large sample of μ -one-charged-hadron events²¹. And physicists using the PLUTO detector at DORIS also found²² such events. As with the $e\mu$ events, all the properties of these μ -hadron events were consistent with the τ being a heavy lepton.

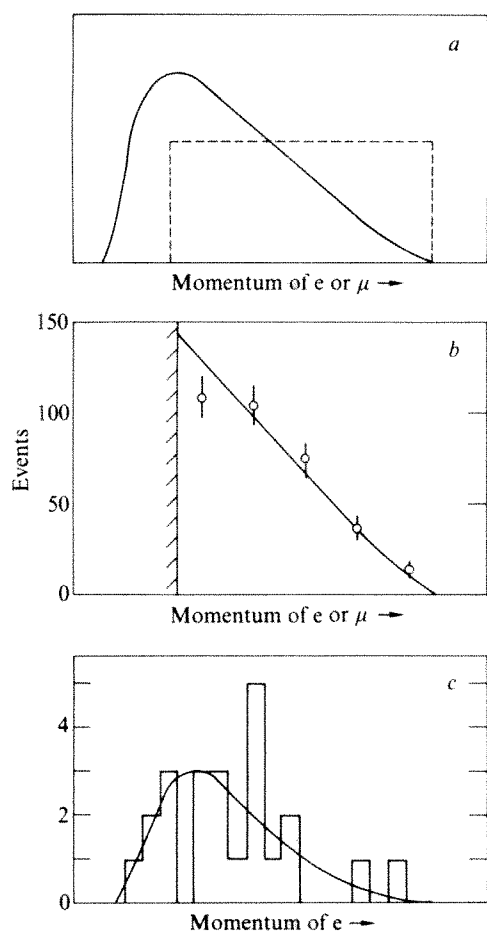
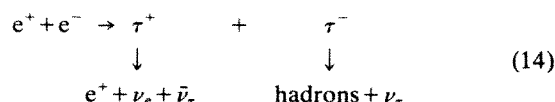


Fig. 4 *a*, The solid curve is the theoretical momentum spectrum of the e or μ coming from the three-body decay $\tau \rightarrow e(\text{or } \mu) + \nu + \bar{\nu}$ of a moving τ . The dashed line is the theoretical momentum spectrum of the e or μ from a two-body decay $\tau \rightarrow e(\text{or } \mu) + \nu$. The comparison of the three-body decay curve with the data is made in (b) for the SLAC-LBL magnetic detector results (ref. 17) and in (c) for the PLUTO detector results (ref. 18). The curves in (b) and (c) are corrected for apparatus acceptance and the spread in E_{total} ; hence they differ slightly from the curve in (a). In (b) the apparatus could not identify e 's or μ 's in the low momentum region marked.

e -Hadron events. The symmetry of the sequential heavy lepton model with respect to τ decays to μ 's and e 's, equation (7), requires that e -hadron events as well as μ -hadron events exist. To find such events it was necessary to have a detector which could precisely separate electrons from hadrons. The DASP detector²³ at DORIS was the first e^+e^- colliding beams detector to have that ability. As shown in Fig. 5, this separation is obtained by using Cerenkov counters. In 1977 the group using DASP was able to find²⁴ the e -one-charged-hadron events expected from the reaction



and more recently physicists using a very large solid angle Cerenkov detector at SPEAR, called DELCO²⁵, have found a very large sample of e -one-charged-hadron events. With the finding of these events, and the determination that their properties were consistent with heavy leptons as their source, the discovery stage of the work on the τ lepton ended. We are now in the stage of detailed study of the τ and measurement of its properties. This is the subject of the next section.

Properties of the τ

Mass of the τ . The most recent measurement of the mass of the τ carried out by the DELCO collaboration²⁶ yields

$$m_\tau = 1,782_{-7}^{+2} \text{ MeV}/c^2 \quad (15)$$

This value was obtained by measuring the cross section, σ_{eh} , for the production of e -one-charged-hadron events as a function of the energy E_{total} . If the τ is a lepton, σ_{eh} should obey the equation

$$\sigma_{eh} = P_{eh} \frac{4\pi\alpha^2}{3E_{\text{total}}^2} \left[\frac{\beta(3-\beta^2)}{2} \right] \quad (16a)$$

Here P_{eh} is the probability that a τ pair decays to an e -one-charged-hadron event; α is the fine structure constant; and $\beta = v/c$ where v is the velocity of the τ and c is the velocity of light. It is conventional in e^+e^- annihilation physics to remove the E_{total}^2 term in equation (16a) by defining the ratio

$$R_{eh} = \frac{\sigma_{eh}}{\sigma_{e^+e^- \rightarrow \mu^+\mu^-}} = P_{eh} \left[\frac{\beta(3-\beta^2)}{2} \right] \quad (16b)$$

Here

$$\sigma_{e^+e^- \rightarrow \mu^+\mu^-} = \frac{4\pi\alpha^2}{3E_{\text{total}}^2} \quad (16c)$$

is the cross section for reaction (4). In equation (16b) as E_{total} increases, β approaches 1 and R_{eh} approaches a constant, namely P_{eh} . Figure 6a shows²⁶ R_{eh} . The E_{total} where R_{eh} begins to rise above zero is called the threshold energy, E_{thresh} , and m_τ is given by $E_{\text{thresh}}/2$. The behaviour of R_{eh} in Fig. 6a and of $R_{e\mu}$ in Fig. 6b are consistent with equation (16b) and hence consistent with the τ being a lepton. ($R_{e\mu} = \sigma_{e\mu}/\sigma_{e^+e^- \rightarrow \mu^+\mu^-}$ where $\sigma_{e\mu}$ is the cross section for producing $e\mu$ events.) An older value of m_τ obtained by the DASP collaboration²⁴ is

$$m_\tau = 1,807 \pm 20 \text{ MeV}/c^2$$

Mass of the ν_τ . The DELCO collaboration²⁵ gives an upper limit of

$$m_{\nu_\tau} \leq 250 \text{ MeV}/c^2$$

with 90% confidence; m_{ν_τ} could be zero. The masses of the e and μ neutrinos may also be zero (Table 1).

Lifetime of the τ . The PLUTO collaboration finds

$$\tau \text{ lifetime} \leq 3.5 \times 10^{-12} \text{ s}$$

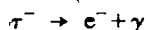
with 95% confidence. The theoretical prediction for this lifetime is $2.5 \times 10^{-13} \text{ s}$.

The coupling of the τ to the ν_τ . The data favour V-A coupling of the τ to the ν_τ . This, of course, is the coupling predicted by conventional weak interaction theory.

Spin of the τ . Measurement of the behaviour of the cross section for $e^+ + e^- \rightarrow \tau^+ + \tau^-$ as a function of E_{total} strongly favours^{3,4,25} the spin of the τ being 1/2. This has been discussed in detail by Tsai²⁷. The spins of the e , μ and their neutrinos are also 1/2, hence the τ on the basis of its spin is a conventional lepton, if we can call a heavy lepton conventional.

Decay modes of the τ . Table 4 lists the observed decay modes of the τ and compares the measured fractional decay rates with the theoretical predictions. These predictions are recent calculations of Tsai¹³ based on conventional weak interaction theory and V-A coupling. In all the measured modes the data and the theory agree; this is another reason for believing that the τ is a lepton.

Lepton type of the τ . All measurements on the τ are consistent with its being a sequential heavy lepton with a unique and separately conserved lepton number. It has been shown^{28,29} that the τ^- does not have the lepton number of the μ^+ or μ^- , nor does it have the lepton number of the e^+ (ref. 30). However, it is possible the τ^- could have the lepton number of the e^- —we have not been able to rule out this possibility experimentally. But this leads to an ugly theory in which the decay



which has not been observed, must be prohibited or suppressed by means other than lepton conservation. My prejudice is that the sequential heavy lepton model, which has guided us so well, will in the end be the correct choice.

Future work on the τ . Studies of the properties of the τ are continuing at SPEAR and DORIS and will also be undertaken at the three new, higher energy, electron-positron colliding beams facilities. These new facilities are PEP at SLAC and PETRA at DESY each with a maximum E_{total} of about 36 GeV, and CESR at Cornell with a maximum E_{total} of about 18 GeV. One direction of these new studies will be to learn more about the decay mode of τ . For example, do decay modes such as $\tau^- \rightarrow K^- + \nu_\tau$ exist at the predicted 1% decay rate? Or does the decay mode $\tau^- \rightarrow e^- + e^+ + e^-$, forbidden by the sequential lepton model, but predicted by other models, exist?

At the higher energy e^+e^- facilities, we can test in more detail our present conclusion that the τ is a lepton. We have come to this conclusion by using Occam's razor and asserting that a charged particle is either a lepton or a hadron. But suppose the τ is some very strange new kind of particle which is 90% leptonic and 10% hadronic in nature. We might expect that the 10%

Table 4 Comparison of predicted and measured fractional decay rates for the τ

Decay mode	Predicted fractional decay rates	Measured fractional decay rates	Groups making measurements
$\tau^- \rightarrow \nu_\tau + e^- + \bar{\nu}_e$ or $\tau^- \rightarrow \nu_\tau + \mu^- + \bar{\nu}_\mu$	16%	16 to 19%	DASP DELCO SLAC-LBL
$\tau^- \rightarrow \nu_\tau + \tau^-$	10%	6 to 11%	DELCO SLAC-LBL PLUTO
$\tau^- \rightarrow \nu_\tau + \rho^-$	23%	24 ± 9%	DASP
$\tau^- \rightarrow \nu_\tau + \geq 3$ charged hadrons	28%	16 to 32%	DELCO MPP PLUTO SLAC-LBL

Where several measurements have been made, the range of values is given. Numbers are rounded off to the nearest per cent. See refs 3, 4 and 25 for details and references.

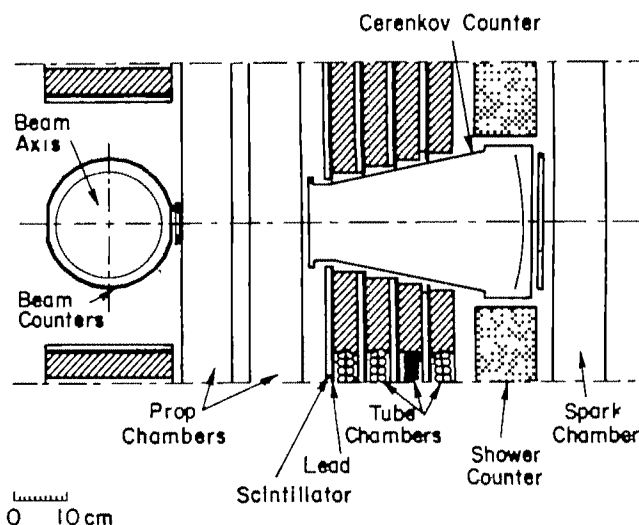
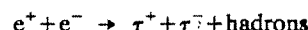


Fig. 5 A section of the DASP detector showing the Cerenkov counter. Redrawn from ref. 23.

hadronic component would show itself at very high energies through the reaction



If, however, the reaction



continues to behave at very high energies as it does at the energies now available, then we shall have additional proof that the τ is a pure lepton.

Leptons and quarks

I conclude this article with two remarks on the possible impact of the discovery of the τ on elementary particle physics. First, we have never understood how to calculate the masses of elementary particles. For example, we do not have the least idea why the mass of the electrons is $0.51 \text{ MeV}/c^2$. Perhaps the discovery of the τ will provide a clue as to how to calculate masses because now for the first time we know the masses of three truly elementary particles: the e , μ and τ . (The neutrinos seem to have zero mass which is not much of a help to calculations.)

Second, as noted above, it has become customary to regard the hadrons as composite particles made up of more elementary particles—the quarks. One then regards the quarks and leptons as two different but similar families of truly elementary particles. Furthermore, in some theories the numbers of leptons are equal to the numbers of quarks. For example, before the discovery of the τ there seemed to be four leptons and four quarks:

Leptons: e, μ, ν_e, ν_μ ; Quarks: u, d, s, c

The discovery of the τ upset this equality. However, it may be restored as follows. If the τ is a sequential heavy lepton, its neutrino, ν_τ , is unique and constitutes a sixth lepton. The discovery of the upsilon particle indicates the existence of a fifth quark called b . If we are then willing to believe that a sixth quark, usually called t , also exists, there is again lepton-quark numerical equality:

Leptons: $e, \mu, \tau, \nu_e, \nu_\mu, \nu_\tau$; Quarks: u, d, s, c, b, t (17)

Many elementary particle physicists would like to see this closed system of twelve elementary particles, with the addition of the intermediate bosons $W^\pm, Z^0$, constitute the fundamental nature of matter. (I am simplifying here. Most theories also require other particles, such as the gluons, which are supposed to carry the forces between the quarks.) After all, such closed systems of elementary constituents have played crucial parts in physics up to now. We explain the long series of atoms as being made of different combinations of electrons around a nucleus; and we

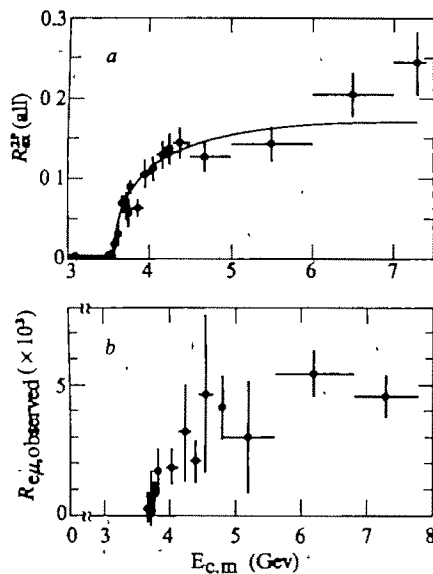


Fig. 6 Behaviour of (a) $R_{\pi^0}^2 = \sigma_{\pi^0}/\sigma_{\pi^0+\pi^0 \rightarrow \mu^+\mu^-}$ from DELCO²⁶; and of (b) $R_{\pi^0}^2 = \sigma_{\pi^0}/\sigma_{\pi^0+\pi^0 \rightarrow \mu^+\mu^-}$ from the SLAC-LBL magnetic detector collaboration^{3,4}.

explain the hundreds of different kinds of nuclei by different combinations of neutrons and protons. If the system of truly elementary particles is closed as in equation (17), then we will also be able to explain the hundred or more kinds of hadrons as various combinations of quarks. And the lepton will remain a relatively simple and sparse family with the τ being the only heavy lepton.

There is an alternative possibility which intrigues me. Perhaps the τ is the beginning of a long, or even infinite, series of heavy leptons; and perhaps these leptons are truly elementary, as the e , μ and τ seem to be. Then the elementary particle physicist will

face a situation unique in physics: a large number of related entities whose properties cannot be explained in terms of a much smaller number of more fundamental entities. Then our present ways of theorising about elementary particles will not work and we will need some radically new ideas. Of course, this is speculation; and for experimenters, there is an obvious task which precedes this speculation. We must find out whether the τ is the first and last of the heavy leptons, or whether it is only the beginning of a sequence of heavy leptons.

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articles

Recurrent activity in galactic nuclei

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Arguments are presented to support the hypothesis that massive galaxies periodically undergo phases of nuclear activity during their evolution at intervals 10^8 – 10^9 yr. The importance of recurrence to theories of active nuclei is emphasised. In particular, the evidence does not favour the production of massive black holes in galactic nuclei.

THEORETICAL investigations into the problems raised by activity in galactic nuclei have, until recently, concentrated almost exclusively on the hypothesis that nuclear activity (as evidenced by the quasar, Seyfert or radio-galaxy phenomena) is

an essentially singular occurrence in the lifetime of a massive galaxy. The possibility that nuclear activity might be a recurrent phenomenon has been considered, but as yet no compelling reasons for preferring one or other hypothesis have been presented. In this article we assess the observational evidence bearing on this question and outline a theory to account for recurrence. Implications of the establishment of recurrence as a fundamental phenomenon affecting the evolution of galaxies are discussed.

Observational evidence for recurrent activity

As there is no general agreement as to either the nature or the evolution of a galactic nucleus, it is still not possible to determine

unambiguously how any one property of a galaxy relates to nuclear activity, recurrent or otherwise. Nevertheless, a past period of activity might be considered to manifest itself in at least one or more of the following ways in normal galaxies: (1) by the existence of a massive nonstellar remnant²; (2) by a burst of star formation in the nuclear region³; (3) by noncircular motions in the nuclear region⁴; (4) by characteristic radio spectra and structures^{5,6}; and (5) by jets⁷ or unusual arms emerging from nuclei^{8,9}. In some instances, the evidence can testify only to at least one period of activity (for example, (1)), or to recent activity (for example, (5)); but in other cases it is possible in principle to compare the observable timescale of a given signature (τ_0) with the cumulative duration (τ) of the phenomenon inferred from its frequency amongst particular galaxy types. The ratio $R = \tau/\tau_0$ is then a measure of the number of events during a galactic lifetime.

If a burst of massive star formation is triggered by a period of nuclear activity—for example, either by immediate condensation of expelled gas or by passage of shocks—the timescale τ_0 may be estimated from the expected lifetimes of a nuclear H II region or the time required for an initial burst to affect the whole nuclear region, to be $\leq 5 \times 10^7$ yr. The central regions of $\sim 10\%$ of spirals show narrow nuclear emission lines associated with a recent burst of star formation^{10–12}, so in this case $\tau \sim 10^9$ yr, giving $R \geq 20$.

Noncircular motions might in principle have a number of separate causes (such as tidal interaction, or action of a bar), but if it is assumed that those observed are due either directly or indirectly to ejected nuclear material⁴, then observable consequences might survive for $\geq 10^8$ yr (ref. 13). The exact timescale must ultimately depend on the nature and viscosity of the interstellar medium, and for the purpose of the present estimate it will be assumed that $\tau_0 \leq 5 \times 10^8$ yr represents a reasonable upper limit. If the sample of galaxies discussed by Burbidge¹⁴ is indeed unbiased (that is, not selected with nuclear activity specifically in mind), then since about half (excluding Seyferts and barred spirals) display noncircular motions $\tau \sim 5 \times 10^9$ yr, and $R \geq 10$. According to the theory discussed by Clube¹⁵ the very presence of spiral arms is an indicator of recently finished nuclear activity, and if this is accepted then the implied value of R is $R \geq 50$.

The radio luminosities of Seyferts, although not usually comparable with those of powerful radio galaxies, are larger than those of normal spirals by factors of $10\text{--}10^3$ (refs 16–18). It is natural to assume that this difference is a consequence of the nuclear activity, and because relativistic electrons radiating at radio wavelengths have synchrotron half-lives of $\leq 10^4$ yr it might be expected that the effects of their decay could be used as a means of distinguishing single from recurrent activity^{5,19}. However, it is difficult to precisely predict the radio evolution of a post-active galaxy, because the result is sensitive to questions such as the time-dependence of the injection spectrum, diffusion losses, and the magnetic field. It is therefore only possible to conclude firmly that the observations are consistent with the hypothesis of recurrence. For radio sources associated with elliptical galaxies, the structural and spectral evidence favours recurrence more directly, with intervals of $< 10^8$ yr (ref. 19) and independent evidence relating to star formation in these galaxies can also be interpreted as evidence for recurrence³.

Our discussion has been confined to apparently normal post-active galaxies. Another estimate of R may be obtained by considering the Seyfert phenomenon itself. This occurs in $\sim 1\%$ of spirals, giving $\tau \sim 10^8$ yr, while the characteristic timescale associated with the velocities ($\sim 10^3$ km s⁻¹) and sizes (~ 1 kpc) of the disturbed central regions of Seyfert galaxies indicate $\tau_0 \sim 10^6$ yr. Thus $R \sim 100$.

We conclude that the available data are not at variance with recurrent nuclear activity, and estimate a mean interval between active periods $\sim 10^8\text{--}10^9$ yr. The analysis below applies particularly to Seyfert activity, the kind most likely to be associated with our own Galaxy. The known properties of our Galaxy can thus serve as a comparatively secure basis for calculation.

The physical basis for recurrence: disk formation

Several authors^{1,3,20} have suggested that material lost from stars undergoing normal stellar evolution represents an inevitable supply of gas from which to fuel nuclear activity. If the stars in the galaxy have any residual angular momentum, the gas will in the first instance flow inwards to form a dense gaseous disk, and the idea that formation of a disk is a necessary step in the creation of an active nucleus has frequently been advanced^{21–23}. If this material is ultimately to collapse into the nucleus it is necessary that it lose both energy and angular momentum; and the long timescales associated with angular momentum transport in astronomical systems²¹ suggest that any theory capable of explaining recurrent nuclear activity must use only stellar mass loss from those relatively low angular momentum stars comprising the bulge components of galaxies.

We first show that this source of gas is sufficient to fuel the Seyfert phenomenon. The stellar mass loss rate from a nuclear star cluster of mass M_n and age t may be shown^{24,25} to be

$$\alpha_* M_n \sim 10^{-2} M_n t^{-1} \quad (1)$$

and adopting $t \sim 10^{10}$ yr and $M_n \sim 10^{10} M_\odot$ (values appropriate for our own Galaxy²⁶), this becomes $\alpha_* M_n \sim 10^{-2} M_\odot \text{yr}^{-1}$. At early epochs ($t \leq 10^9$ yr, say) the mass loss rate is slightly higher than that given by (1), and a more detailed calculation²⁷ shows that the total fractional mass loss during 10^{10} yr is of the order of 10%. The cumulative stellar mass loss in $\sim 10^{10}$ yr in the nuclear region of our Galaxy ($r \leq 1$ kpc) is therefore $\sim 10^9 M_\odot$. The total mass-energy required by the Seyfert phenomenon in this time may be estimated to be $E_s \sim 2 \times 10^6 M_\odot c^2$ (assuming a typical luminosity $L \sim 10^{38}$ W and a cumulative duration for the Seyfert phenomenon of the order of 10^8 yr), so even if energy is released at only 'nuclear' efficiencies ($\sim 0.7\%$) the postulated source of gas is still sufficient to fuel Seyfert activity.

An important objection to a model in which nuclear activity is fuelled by stellar mass loss is that supernovae in the nuclear star cluster might heat the gas up to such high temperatures that a steady outflowing galactic wind would occur²⁸. It should be emphasised that this conclusion is only valid provided that heating dominates cooling, and that the system contains no sink²⁹. In the present problem it has been shown³⁰ that steady winds are only favoured in elliptical galaxies at epochs $t \geq 10^9$ yr, with the flow at earlier times being thermally unsteady and directed inwards. The question as to what sort of flow is eventually achieved depends crucially on what happens at time $t \sim 10^9$ yr, since then (when a steady wind first becomes favoured) the flow will initially be inwards. If the disk formed by inflowing gas at early epochs is sufficiently massive and dense it will be able to absorb even hot inflowing gas without heating up, and the appropriate solution to the galactic wind equations will then be one including the presence of a massive disk-like sink. Even if supernovae heat the stellar mass loss with high efficiency the flow can continue inwards, and provided that the sink is strong enough we conclude that outflowing galactic winds do not remove material from the central regions of galaxies.

Physical state, structure and evolution of the disk

To account for the observed nuclear activity it is necessary first that the disk survives as a sink, even when (possibly) hot material is falling on to it; and second that the disk evolves in such a way as to store gas for long periods until a sufficient mass has accumulated that a period of nuclear activity might be produced. To verify that these conditions can indeed be met for a very simple model (more detailed arguments are given elsewhere²⁵), it is assumed that the disk has a surface density

$$\sigma(\tilde{\omega}) = \frac{M_d}{2\pi R_d} \frac{1}{\tilde{\omega}} \cos^{-1}(\tilde{\omega}/R_d) \quad (2)$$

where M_d and R_d are the disk mass and radius respectively. It

may be shown that this density distribution would arise in a model in which the disk is formed out of stellar mass loss from a star cluster with density defined (compare ref. 26) by

$$\rho_*(r) = \frac{M_n}{4\pi R_n} \frac{1}{r^2} \quad (3)$$

and in which it is assumed that the net rotational velocity of the stars at equatorial radius $\tilde{\omega}$ is given by $V_{\text{rot}}(\tilde{\omega}) = \text{constant}$. A model with the same star density but uniform rotation would give a somewhat more centrally condensed disk, but the essentials of the argument do not depend on this detail.

If the r.m.s. gas velocity in the disk is V_s , it may be shown^{31,32} that the disk width $H(\tilde{\omega})$, defined as

$$H(\tilde{\omega}) = \sigma(\tilde{\omega}) / \rho(\tilde{\omega}, 0)$$

is given by

$$H(\tilde{\omega}) = \zeta \frac{V_s}{V_0} \tilde{\omega} \quad (4)$$

where ζ is a numerical factor which, for disks that are gravitationally stable, is of order unity, and V_0 is the (constant) circular velocity in the model: $V_0 = (GM_n/R_n)^{1/2}$. The mid-plane particle density is therefore

$$n(\tilde{\omega}, 0) \sim 3 \times 10^9 \left(\frac{M_d}{10^7 M_\odot} \right) \left(\frac{100 \text{ pc}}{\tilde{\omega}} \right)^2 \times \left(\frac{\cos^{-1}(\tilde{\omega}/R_d)}{\zeta} \right) \left(\frac{1 \text{ km s}^{-1}}{V_s} \right)^2 \text{ m}^{-3} \quad (5)$$

where the mean particle mass has been taken to be $m_H \sim 1.7 \times 10^{-27} \text{ kg}$ and the values $M_n \sim 10^{10} M_\odot$, $R_n \sim 1 \text{ kpc}$ and $R_d \sim 500 \text{ pc}$ have been assumed. The disk is thus very dense, even if V_s should be several tens of km s^{-1} , and it may be expected that cooling, proportional to n^2 , will be efficient. In order to estimate the equilibrium temperature of the disk we consider the balance between heating and cooling processes, as represented by the equation

$$\Gamma = n^2 \Lambda(T)$$

where Λ is the usual cooling function and Γ is the power input per unit volume from various sources.

Four sources of heating have been considered as of possible importance for the energetics of the disk: (1) heating due to possibly hot infalling material, (2) heating by stars located within the disk, (3) heating by supernovae within the disk, and (4) heating by the effect of stars passing through the disk. On the assumption that stellar mass loss does not lead to further star formation until the disk becomes gravitationally unstable (so that until the point is reached the system contains no massive O-B stars), it may be shown that heating is dominated by hot infalling material. This heat source may be written in the form

$$\Gamma_1 \sim \epsilon(\tilde{\omega}) \frac{\partial \rho}{\partial t}$$

where ϵ is the excess energy deposited by unit mass entering the disk and $\partial \rho / \partial t$ is given approximately by $1/H \partial \sigma / \partial t$. The energy carried by unit mass entering the disk consists of a thermal part $\epsilon_{\text{th}} \sim 3kT/m_H$, and a gravitational part $\epsilon_{\text{grav}} \sim V_0^2$; so Γ_1 is given approximately by

$$\Gamma_1 \sim \left(\frac{3kT}{m_H} + V_0^2 \right) \frac{1}{H(\tilde{\omega})} \frac{\partial \sigma}{\partial t} \quad (6)$$

By substituting for H and $\partial \sigma / \partial t$ (making use of $(dM_d/dt) = \alpha_* M_n$), and assuming that the infalling material has an initial

temperature $T \sim 2 \times 10^7 \text{ K}$ corresponding to that of a steady supernova-heated flow²⁸, this reduces to

$$\Gamma_1 \sim 7.5 \times 10^{-23} \left(\frac{\alpha_* M_n}{10^{-2} M_\odot \text{ yr}^{-1}} \right) \left(\frac{500 \text{ pc}}{R_d} \right) \times \left(\frac{\cos^{-1}(\tilde{\omega}/R_d)}{\zeta} \right) \left(\frac{1 \text{ km s}^{-1}}{V_s} \right) \left(\frac{100 \text{ pc}}{\tilde{\omega}} \right)^2 \text{ W m}^{-3} \quad (7)$$

Since $\Gamma \sim \Gamma_1$, combining (7) with $n \sim n(\tilde{\omega}, 0)$ gives the value of the cooling function required to just keep the disk at temperature T . That is,

$$\Lambda_{\text{req}} \sim \Gamma_1 / n^2(\tilde{\omega}, 0) \Rightarrow \Lambda_{\text{req}} \sim 10^{-41} \left(\frac{\alpha_* M_n}{10^{-2} M_\odot \text{ yr}^{-1}} \right) \left(\frac{V_s}{1 \text{ km s}^{-1}} \right) \times \left(\frac{10^7 M_\odot}{M_d} \right)^2 \left(\frac{\tilde{\omega}}{100 \text{ pc}} \right)^2 \text{ W m}^3 \quad (8)$$

where as before it has been assumed that $M_n \sim 10^{10} M_\odot$, $R_n \sim 1 \text{ kpc}$ and $R_d \sim 500 \text{ pc}$, and factors of order unity have been dropped. Comparing this with the cooling function appropriate for a lightly ionised atomic disk³³ and assuming that $V_s \sim V_{\text{sound}}$ (since strongly supersonic motions are quickly damped), implies the disk will cool—especially in its central regions—to a temperature $\leq 50 \text{ K}$. It is thus concluded that a representative disk of mass $M_d \sim 10^7 M_\odot$ will be cold, and in its central regions will consist of gas mainly in molecular form. The disk therefore does act as a sink, and is able to absorb all the infalling gas due to stellar mass loss without heating up.

If viscous evolution may be neglected during this phase of the disk's evolution (this is verified below), the disk will slowly grow in mass until the onset of gravitational instability. The critical surface density may be shown³¹ to be given approximately by

$$\sigma_{\text{crit}} \sim \frac{\kappa V_s}{\pi G} \quad (9)$$

where κ is the epicyclic frequency, given by $\sqrt{2}(V_0/\tilde{\omega})$ in the present model. Substituting $\sigma(\tilde{\omega})$ into (9) gives the critical mass at which instability first occurs at radius $\tilde{\omega}$:

$$M_{\text{crit}}(\tilde{\omega}) \sim 2\sqrt{2} \left(\frac{V_s}{V_0} \right) \left(\frac{R_d}{R_n} \right) \frac{M_n}{\cos^{-1}(\tilde{\omega}/R_d)} \quad (10)$$

Use of the previously adopted values of M_n , R_n and R_d thus shows that the disk will first fragment close to the origin when its mass reaches

$$M_{\text{crit}} \sim 4 \times 10^7 \left(\frac{V_s}{1 \text{ km s}^{-1}} \right) M_\odot \quad (11)$$

The onset of gravitational instability in the disk is expected to lead to a burst of star formation, and if some of the newly-formed stars are sufficiently massive to drive strong turbulence in the disk, the associated viscosity will lead to large central flows of matter. The inwards flux of matter across radius $\tilde{\omega}$ may be shown²¹ to be given approximately by

$$F \sim 2\pi\nu\sigma \quad (12)$$

where ν is the kinematic viscosity. This is given roughly by $\nu \sim \frac{1}{3}\bar{c}l$ where $\bar{c}$ is the mean velocity of a turbulent element and l its mean free path²¹. If l is taken to equal the mean epicyclic amplitude of cloud motions, $l \sim \bar{c}/\kappa$, the kinematic viscosity becomes

$$\nu \sim \frac{1}{3\sqrt{2}} \frac{\bar{c}^2}{V_0} \tilde{\omega} \quad (13)$$

The expected central flux of matter just after the onset of gravitational instability is thus

$$F_{\text{acc}} \sim 2\pi r v_{\text{acc}} \sim \frac{2}{3} \frac{\bar{c}^2 V_s}{G}$$

$$\Rightarrow F_{\text{acc}} \sim 0.06 \left(\frac{\bar{c}}{20 \text{ km s}^{-1}} \right)^2 \left(\frac{V_s}{1 \text{ km s}^{-1}} \right) M_{\odot} \text{ yr}^{-1} \quad (14)$$

where $\bar{c} \sim 20 \text{ km s}^{-1}$ has been assumed representative of the mean turbulent velocity in a newly-formed H II region. The flux just before gravitational instability may be estimated from this formula by setting $\bar{c} \sim V_s \sim 1 \text{ km s}^{-1}$, thereby verifying that viscous flows during the quiescent phase of the disk's evolution are indeed small.

If nuclear activity is produced by a period of rapid accretion on to a massive black hole^{2,23,34} energy may be produced with an efficiency, η , approaching 30% (ref. 35). The luminosity generated by such a process is given by

$$L \sim \eta F_{\text{acc}} c^2$$

$$\Rightarrow L \sim 10^{38} \left(\frac{\eta}{0.3} \right) \left(\frac{\bar{c}}{20 \text{ km s}^{-1}} \right)^2 \left(\frac{V_s}{1 \text{ km s}^{-1}} \right) \text{ W} \quad (15)$$

which is very close to the observed luminosities of typical Seyfert galaxies³⁶. From this result we conclude that although other theories of the nature of nuclear activity might be more appropriate (see the discussion), the theory described here is not inconsistent with accretion on to a black hole. Observations of Seyfert galaxies indicate the presence of substantial masses of gas in the nuclear regions^{37,38}, so it is unlikely that normal nuclear activity is able to totally destroy the disk and remove all the gas from the central region. The disk (or parts of it) therefore survives a period of Seyfert activity and the processes described above can be expected to recur, thereby producing a mechanism by which recurrent nuclear activity might be understood.

We note in passing that although conceivable, steady evolution of the disk does not seem likely. For example, it is possible to envisage a steady state in which infall is just balanced by a slow rate of star formation. However, such a situation could only be truly steady if the disk always formed low-mass stars, a possibility that we regard unlikely. The occurrence of even a few massive stars would lead to more³⁹, and eventually turbulent velocities large enough to cause inward fluxes much greater than the infall rate (and sufficient to lead to a period of nuclear activity) would be generated. Massive star formation leads to non-steady evolution. In a different kind of steady state it might be argued (compare ref. 22) that once gravitational instability³¹ has occurred, the heat generated by cloud-cloud collisions and viscous friction could be large enough to maintain a relatively large velocity dispersion in the disk. A steady inward transport at a rate equal to the stellar mass loss rate might then occur, and recurrence (at least on the black hole model) would not necessarily follow. In the present analysis the inward flux immediately after gravitational instability is F_{acc} , implying the viscous heating rate per unit area is given²¹ by

$$D(\tilde{\omega}) \sim F_{\text{acc}} V_0^2 / 2\pi \tilde{\omega}^2$$

Dividing this by the disk width (equation (4)) for a disk with velocity dispersion characterised by turbulent velocities $\bar{c} \gg V_s$ (where V_s is the velocity dispersion just before gravitational instability) gives the volumetric heating rate due to viscous processes, Γ_{visc} :

$$\Gamma_{\text{visc}} \sim \frac{1}{3\pi \zeta} \frac{V_0^2 \bar{c} V_s}{\tilde{\omega}^3 G}$$

that is,

$$\Gamma_{\text{visc}} \sim \frac{5 \times 10^{-24}}{\zeta} \left(\frac{\bar{c}}{10 \text{ km s}^{-1}} \right) \left(\frac{V_s}{1 \text{ km s}^{-1}} \right) \left(\frac{100 \text{ pc}}{\tilde{\omega}} \right)^3 \text{ W m}^{-3} \quad (16)$$

where the usual values of M_0 and R_0 have been assumed. Comparing this expression with (7) shows that viscous heating is unimportant to the energy balance of the disk at $\tilde{\omega} \geq 10 \text{ pc}$, and even at $\tilde{\omega} \sim 1 \text{ pc}$ is only about five times larger than Γ_1 (for $V_s = 1 \text{ km s}^{-1}$ and $\bar{c} = 10 \text{ km s}^{-1}$). The conclusion that the disk will be very cold therefore remains valid, and unless some other energy source intervenes (such as massive star formation) turbulent velocities large enough to drive inward flows of order $\alpha_* M_* \sim 10^{-2} M_{\odot} \text{ yr}^{-1}$ will not be produced. If stars are formed this case reduces to the one above, and steady evolution can only occur if no massive stars form.

Discussion

In the previous sections it has been shown that not only do observations strongly support the idea that nuclear activity in massive galaxies (such as our own) is a recurrent phenomenon, but also this might be rather simply understood by a model in which stellar mass loss leads periodically to nuclear activity via the intermediary of a dense cold (probably molecular) gaseous disk. Arguments based on the rate of viscous transport in the central regions of the disk and the expected evolutionary time-scale of any massive object likely to be formed ($\tau_{\text{evol}} \sim 10^6 \text{ yr}$, say) lead to the conclusion that in the first instance a body with mass in the approximate range 10^4 – $10^6 M_{\odot}$ will be formed (for example, equation (14)). Depending on the detailed evolution of a body of this size, three possibilities may be distinguished.

In the first, it is assumed^{2,40} that any body much more massive than an ordinary star will end its evolution by a period of relativistic collapse, ultimately forming a massive black hole. However, the present theory (based on parameters thought to be typical of our own Galaxy) combined with the observations that the galactic centre does contain large amounts of gas, implies that our Galaxy has undergone recurrent periods of nuclear activity; this is also consistent with the conclusion reached by Lynden-Bell², that the observed numbers of quasars makes it difficult to argue that our Galaxy has never been active. If our Galaxy was active in the distant past, the expected size of the black hole now in its nucleus may be estimated in several ways. First by comparing typical Seyfert or quasar powers with the Eddington limit of a mass M_b ; for $L_{\text{av}} \sim 10^{38} \text{ W}$ this gives $M_b \approx 5 \times 10^6 M_{\odot}$, with much larger masses being indicated if our Galaxy has ever been as luminous as a quasar⁴¹. Second, by dividing E_{av} by ηc^2 , where η may be estimated³⁵ to be ≤ 0.3 ; this again gives $M_b \approx 5 \times 10^6 M_{\odot}$. Third, by assuming growth at an average rate limited by the stellar mass loss rate²³. With $\alpha_* M_* \sim 10^{-2} M_{\odot} \text{ yr}^{-1}$ this gives $M_b \sim f \times 10^6 M_{\odot}$, where f is the unknown fraction of the mass lost by stars actually transported into the origin; and lastly by assuming growth at a rate limited by the stellar tidal disruption rate^{42–44}, giving $M_b \sim 10^4 M_{\odot}$ provided that the initial hole mass exceeded $\sim 10^6 M_{\odot}$. (Lower mass holes do not grow rapidly in normal galactic nuclei by this process, and the estimate is particularly uncertain as it is possible that the net accretion rate may be much smaller than the tidal disruption rate^{45,46}.) Observationally, current data can be understood without appeal to exotic objects such as black holes^{47,48}, and estimates of the mass within a radius of $\sim 1 \text{ pc}$ ($\sim 4 \times 10^6 M_{\odot}$) are consistent with it being mostly in stellar form^{49,26}. Since observational upper limits to the black hole mass are less (albeit close to) than the theoretical lower limits, we tentatively conclude that black holes are not usually formed in galactic nuclei.

This leaves one of two possibilities. Either the body formed at the centre of the disk explodes disruptively when nuclear burning is initiated^{50,51}, or the body does enter a period of relativistic collapse, but does not form a massive black hole. General relativity has yet to be tested experimentally in the relativistic regime, so this possibility should perhaps not be too lightly dismissed on the ground that it invokes 'new physics'. A detailed discussion of this case is given by Clube¹⁵, who argues that a wide range of independent observations of our Galaxy can be understood most simply on a model in which the Galaxy periodically grows a nucleus whose late evolution does require new physics for its explanation. Here we consider the more conventional

possibility, and note that although the evolution of low-mass supermassive stars as described by Fricke and others does not seem capable of explaining the high luminosities of Seyferts and quasars (approaching in some cases up to $\sim 10^{41}$ W), the nonthermal radiation of a spinar can in some circumstances far exceed the thermal (Eddington-limited) component⁵². It seems likely therefore that the disk will in the first instance form a rapidly rotating disk-like configuration at its centre—resembling a flattened spinar—and although spinar masses are usually assumed to be $\geq 10^8 M_\odot$ (to explain the large energies required by active nuclei for single events), adopting recurrence would lower this requirement considerably. The repeated formation and evolution of low-mass spinars ($M \leq 10^6 M_\odot$) might well explain most nuclear activity, although until the effect on the object of nuclear burning is better understood (that is, whether or not leading to a disruptive nuclear explosion) it is not possible to say with certainty whether 'new physics' is required by the data. It should be noted that stellar mass loss can explain Seyfert energetics even if energy is produced only at nuclear efficiencies.

Thus we have shown here that a wide variety of observational evidence indicates that nuclear activity is a recurrent phenomenon, and that this might be understood on a theory in which activity is caused by gas lost from stars evolving to form an active nucleus via the intermediary of a dense gaseous disk. Following Ozernov and Usov⁵² we conclude that recurrent nuclear activity is best understood by a spinar model, although the present theory indicates that in the first instance only low-mass objects ($M \leq 10^6 M_\odot$) are likely to be formed. Calculation of the late evolution of such a mass depends critically on the effects of the onset of nuclear burning, and our current understanding of this problem does not allow the possibility of gravitational collapse to be distinguished theoretically from that of a disruptive nuclear explosion. From the observational viewpoint the formation of massive compact relativistic objects seems extremely probable⁵³, with one of their most important characteristics being the ejection of both matter and energy⁵⁴.

The hypothesis of recurrence places severe constraints on theories of the origin of nuclear activity. In particular, the apparent lack of a supermassive black hole in our galactic centre argues against the black hole theory, although it should be emphasised that this conclusion does not affect the discussion in the main part of the paper. Because of its potential to discriminate between theories of nuclear activity, further work aimed at distinguishing recurrence from single activity should be given high priority.

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Re-appraisal of lithostratigraphy of Sterkfontein hominid site

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A new lithostratigraphic classification is proposed for the Sterkfontein hominid site. The Sterkfontein Formation is defined in terms of constituent members and beds. The new terminology is correlated with that used previously and, where possible, hominid finds are linked to the newly defined lithostratigraphic units.

THE stratigraphy of the rich site at Sterkfontein, where *Australopithecus africanus* was discovered in 1936, has been investigated several times. Between 1938 and 1952 several

broad descriptive classifications of the deposits were proposed^{1–4}. The first detailed stratigraphic and sedimentological analysis appeared in 1958 (ref. 5); this supplemented by a further generic re-appraisal of portions of the succession⁶, has provided the standard lithostratigraphic subdivision of the deposit until recently. A subsequent geomorphological study of the Sterkfontein cave system⁷ included some deposits. A re-appraisal of the lithostratigraphy of the hominid deposit is necessary because earlier stratigraphic studies did not examine its full vertical extent; the application of contemporary sedimentological measures and stratigraphic classification procedures also seems desirable. In this article I call the hominid-bearing deposit the Sterkfontein Formation.

Location and origin

The Sterkfontein Formation is the largest continuous cave filling at the site ($26^{\circ} 01' S$, $27^{\circ} 44' E$), and is vertically and laterally continuous through both the Type and Extension sites. It has been exposed both at surface and in the underlying cave system by excavations at elevations of 1,460–1,480 m. At surface the deposit measures approximately 75×15 m. It is exposed on the southern flank of the Sterkfontein hill, the top of which is about 2 m above the highest exposure.

The formation occurs in disconformable juxtaposition with the Malmani Dolomite⁸ of the Transvaal Supergroup, having accumulated within a karst cavity. The sedimentary sequence has been modified periodically by subsidence into underlying cavities formed or enlarged after accumulation of the deposits, by collapse of the cavern walls and roof, by the introduction and dissolution of calcium carbonate cement, and by bevelling of the succession by erosion of the valley flanks of the Bloubaank river (formerly Blaaubank). The Malmani Dolomite here consists of an interbedded succession of dolomite and chert horizons.

The formation of the original solutional receptacle and its subsequent morphological development conform closely with the model of karst development under a spasmodically falling water table proposed for the Transvaal⁹. This initial cavern owed its form to phreatic solution, but was modified by enlargement and collapse in the vadose zone; these modifications were often contemporaneous with the accumulation of the cave filling. The form of the receptacle was controlled by preferential solution along joints and small faults and within locally more soluble horizons in the dolomite, which here dips at approximately 30° NNW. The water table occurs in the underground cave system below the base of the Sterkfontein Formation at an elevation of approximately 1,440 m. Deposits postdating the Sterkfontein Formation are discontinuously present from surface down to this level, and, in places, display complex disconformable relationships with certain of its members.

Although relatively extensive areas of dolomite roof are preserved over parts of the deposit, much of its surface has been bevelled by erosion of the hillside. The inclinations of the lower surfaces of roof segments conform with the local dip of the dolomite bedding planes; a projection of these remnants gives an indication of the maximum elevation of the original roof, which was probably situated some 10 m above the southern part of the deposit. Hence the absolute thickness of the deposit may have exceeded 30 m, less than 70% of which is still preserved.

The original floor of the receptacle varied considerably in elevation; the thickness of the various sedimentary members is very variable and the irregular shape of the depository and obstructing roof pendants produced local variations in the direction of sediment transport. Stratigraphic relationships are consequently complex, and the schematic column in Fig. 1 merely reflects the relative average thicknesses of the various units in accessible areas of the deposit.

Lithostratigraphy

Laboratory analyses were made of large samples (> 5 kg) from average vertical separations of < 2 m, recovered, as far as possible, from three sampling areas. The procedure involved colour identification using Munsell soil colour charts, removal of CaCO_3 in 10% cold dilute HCl, wet sieving and hydrometer analysis of the residue between 1 and $2,000 \mu\text{m}$ at average mesh size intervals of 0.5ϕ (using sodium hexametaphosphate as a peptising agent), plotting of cumulative textural curves, calculation of Trask indices and statistical parameters using the formulae of Folk and Ward¹⁰, examination of the medium sand residue under a $\times 40$ binocular microscope, and X-ray diffraction analysis of the $-75\text{-}\mu\text{m}$ particle size fraction from selected samples.

The very coarse (> 2 mm) fraction of all units was examined visually, and consists chiefly of subangular fragments ranging from pebble to boulder size. The smaller (pebble to cobble) fraction predominates in Members 2 and 6, while large cobbles

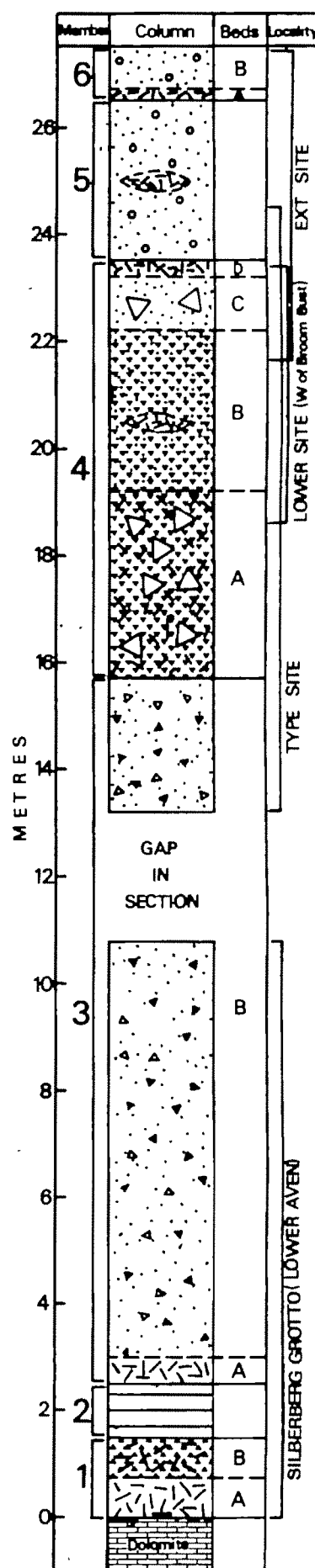


Fig. 1 Stratigraphic column of the Sterkfontein Formation (see text for details)

and boulders are characteristic of bed C of Member 4. In the case of Members 2 and 6 these coarse elements consist almost exclusively of chert, which has sometimes suffered extensive post-depositional snuffbox weathering. In the other units both chert and dolomite are represented; individual fragments, particularly in the larger fractions, are mostly fresh, but may show thin weathering skins on one or two faces, indicating that they accumulated through intracavernous collapses of the roof and projecting ledges. The finer fractions frequently include subrounded chert elements, which are more uniformly and deeply weathered; these represent an extracavernous colluvial component of the deposit. Only in bed B of Member 4 does weathered dolomite contribute significantly to these colluvial elements.

Member 1 is exposed above the weathered dolomite floor in Silberberg Grotto. It consists of 0.5–2.0 m of white banded pale brown (10 YR 7/3) recrystallised meso- and macrocrystalline calcite, locally contaminated with fine clastic sediment. The lower 50%, constituting bed A, has little contamination and no bone. Bed B includes numerous angular chert and dolomite pebbles and cobbles and occasional bone fragments, indicating a small surface opening. The upper contact is wavy and gradational.

Member 2 is exposed in Silberberg Grotto. It consists of 0.5–5.0 m of light brown and red (7.5 YR 6/4–2.5 YR 5/6), mostly centimetre-bedded silty loam (slightly clayey sand-silt) containing numerous broken speleothems, occasional calcite lenses, scattered pebbles and cobbles, and high concentrations of bone near the base. Deposition has occurred around a large stalagmitic boss belonging to Member 1; to the east, bedding planes dip approximately 10° SE, while to the west, the sediments comprise a steep debris cone. CaCO₃ averages 73% and average ϕ mean is 5.20. Sediments are poorly to very poorly sorted¹¹ and highly leptokurtic with pronounced negative skewness. Crystalline minerals (in decreasing abundance) are quartz, pyrophyllite, sericite, goethite and kaolinite. Typical particulate roundness and sphericity are 0.1 and 0.5 respectively, indicating higher angularity than is characteristic of present external colluvial soils. As in all succeeding units, quartz particles predominate; chert is present in very small proportions and dolomite grains are rare. Surface staining is largely absent. Contact is slightly wavy and abrupt.

These features suggest periodic ponding and episodic subaqueous deposition with flushing of some fines into more distant depositories to the east of the boss, and conical gravitative accretion to the west. Sedimentary parameters suggest higher energy conditions near the middle of the bed. Distribution of this and succeeding members, and direction of depositional dip relative to relief in cave floor, indicate the presence of an opening vertically above the eastern end of Silberberg Grotto.

Member 3 consists of beds A and B. Bed A is exposed in Silberberg Grotto and is composed of recrystallised mesocrystalline calcite averaging 0.5 m thick and dipping about 15° SE. Bed B is exposed in Silberberg Grotto and the East Pit of the Type Site (top of bed only). It consists of about 10 m of reddish brown (5 YR 5/4) occasionally speckled red (5 YR 5/8) loam (clayey silty sand) containing scattered angular pebbles, cobbles and boulders and abundant bone fragments. Bedding is not discernible, but calcite lenses occur in lower levels. CaCO₃ averages 76% and average ϕ mean is 5.10. Sediments are very poorly sorted, leptokurtic and very positively skewed. Crystalline minerals are quartz, goethite, sericite, feldspar and kaolinite. Typical roundness and sphericity are 0.3 and 0.5 and compare with outside colluvium. There is occasional pyrolusite staining. Contact is apparently conformable and abrupt. The sedimentology indicates slow, uniform gravitative accretion of external colluvium and, possibly, some aeolian sand through small openings without significant resorting by water, but with occasional collapses of protruding cave ledges.

Member 4 consists of beds A, B, C and D. Bed A is exposed in the Type Site and the Lower Cave. It consists of 1–5 m of

angular pebbles, cobbles and boulders cemented by recrystallised meso- and macrocrystalline calcite, containing occasional small irregular pockets of reddish brown (5 YR 5/4) silty loam (slightly clayey silty sand) and occasional bone fragments. Fine sediments were apparently contemporaneous with the coarse fraction, and are similar to those of bed B, with a ϕ mean of 4.90. Contact is indigitating. This bed indicates a series of major roof collapses in rapid succession, greatly enlarging the cave opening. This is supported by analysis of the ¹³C content of the calcite within succeeding units; a significant increase in ¹³C above this level suggests greatly improved ventilation of the cave¹².

Bed B is exposed in the Type Site, the Lower Cave and lower levels of the Extension Site. It consists of 1.0–5.5 m of predominantly reddish brown (5 YR 4/4 and 5/4), but less frequently yellowish red (5 YR 5/6) and red (2.5 YR 4/6 to 5/8) sandy loam (slightly clayey silty sand) containing abundant pebbles, cobbles and boulders and occasional bone fragments. Crude stratification is present in the very coarse fraction, parallel to the flanks of a debris cone with its apex near the southern margin of the Type Site excavation. Infrequent recrystallised calcite lenses occur on the flanks of the cone. CaCO₃ averages 53% and average ϕ mean is 4.47. Sediments are poorly to very poorly sorted and leptokurtic with a wide range of skewness from negative to positive. Near the cone periphery (for example, in the Lower Cave) sediments are less negatively skewed and less markedly leptokurtic. Crystalline minerals are quartz, goethite, sericite and kaolinite. Typical roundness and sphericity are 0.3 and 0.5, and particles often show pyrolusite staining. Contact is eroded and disconformable.

The characteristics of this bed are consistent with discontinuous redistribution of gravelly colluvium, in admixture with roof collapse debris, down the flanks of a cone of gravitative accretion under the influence of sheetfloods entering through an enlarged cave opening. Higher energy conditions are evident towards the middle of this unit, especially around the periphery of the cone.

Bed C is exposed in several large, isolated pockets in the Type Site and the Lower Cave. It consists of 0.5–2.0 m of yellowish red (5 YR 5/6) or red (2.5 YR 4/8) unbedded sandy loam (slightly clayey silty sand) containing occasional angular cobbles and boulders and localised concentrations of bone. CaCO₃ averages 53% and average ϕ mean is 4.11. Sediments are very poorly sorted and mesokurtic to leptokurtic with marked negative skewness. Crystalline minerals are quartz, sericite, kaolinite and goethite. Typical roundness and sphericity are 0.3 and 0.5, and pyrolusite staining is common. Contact is even and abrupt.

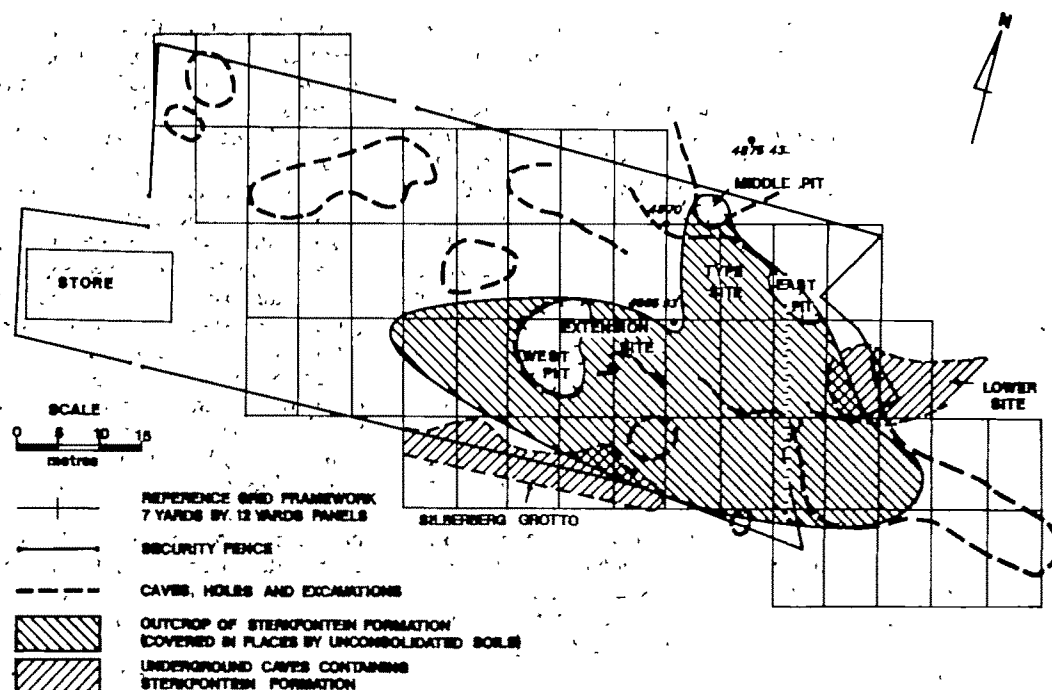
There seems to have been greater dominance of water action than in bed B, with flushing out of fines. The gravelly component of the external colluvium (if present) was not introduced during

Table 1 Comparative terminology of Sterkfontein Formation

Terminology		Hominid remains
Previous (Robinson ⁶)	Present	
Upper breccia (Extension Site)	Member 6	—
Middle breccia (Extension Site and uppermost Type Site)	Member 5	StW 53 and stone industry
Lower breccia (Type Site, Lower Cave and base of Extension Site)	Member 4 {	StS5*, StW 12, 13, 14 & 17 StS 17*, 71* The remainder of the specimens of <i>A. africanus</i> were recovered either from bed B or bed C
	{ Bed C Bed B	

* Correlation inferred from Broom *et al.*'s²² description of host deposits.

Fig. 2 Locality plan showing the surface extent of the Sterkfontein Formation and the positions of localities mentioned in the text. (After A. R. Hughes.)



this period; and intracavernous collapses were less frequent. Higher energy conditions prevailed during deposition in the middle of this unit.

Bed D is exposed in the Type Site and the Lower Cave, vertically above pockets of bed C. It consists of discontinuous white, to brownish (10 YR 5/3) horizontally laminated, recrystallised meso- to macrocrystalline calcite lenses up to 75 cm thick. These consist of accumulations of thin calcite rafts which occasionally form in pools in contemporary Transvaal caves. Contact is unconformable and eroded; there has been minor subsidence of Member 4 before accumulation of Member 5.

This bed represents a period of greater abundance of water in the cave. The presence of very large collapse blocks incorporated in the calcite cement indicates another episode of roof instability.

Member 5 is exposed at the Extension Site and the western end of the Type Site. It consists of 1–5 m of reddish brown (5 YR 5/4 and occasionally 4/4) sandy loam (slightly clayey silty sand) containing numerous pebbles, cobbles and boulders, mostly of chert and dolomite, but including foreign quartz, quartzite and diabase, which are believed to have been introduced by hominids¹³. Bone is occasionally present, and a few calcite lenses occur near the middle of the member. Crude bedding, mostly conformable with the eroded surface of Member 4, is evident in the very coarse fraction. CaCO_3 averages 54% and average ϕ mean is 3.85. Sediments are very poorly sorted and meso- to leptokurtic with negative skewness. Crystalline minerals are quartz, goethite, feldspar, sericite and kaolinite. Roundness and sphericity are similar to those in Members 3 and 4, but pyrolusite staining is less frequent. Contact is abrupt, eroded and unconformable.

The mode of accumulation was as in bed B of Member 4, but without the introduction of weathered dolomite colluvial gravels. Differences in the range of skewness can be ascribed to the relative positions of exposures within the depositional cone. The configuration of crude stratification confirms the location of the main cave entrance to the south of the Type Site. Evidence of cyclically higher energy conditions is present near the middle of this member.

Member 6 is exposed on the north wall of the West Pit excavation in the Extension Site. It consists of beds A and B. Bed A is composed of up to 25 cm of contaminated and recryst-

allised basal calcite, which is occasionally bone-bearing. Bed B is 0.5–1.0 m of dark reddish brown (5 YR 3/3) sandy loam (clayey silty sand) containing occasional pebbles and cobbles, including foreign stone and some bone. A single sample from bed B had a CaCO_3 content of 47% and a ϕ mean of 5.10. Sediments are very poorly sorted out and highly leptokurtic with positive skewness. Crystalline minerals are quartz, sericite, kaolinite, goethite and feldspar. Roundness and sphericity are 0.5 and 0.7 and a large proportion of particles shows pyrolusite staining.

Bed B is similar to the pebbly colluvial soils which now occur on adjacent areas of the Sterkfontein hill; however, foreign stone is absent from the latter, and sand grains of Member 6 are less angular.

Extracavernous source materials

Comparative analyses of deposits from the vicinity indicate that the –2-mm fraction of the various units was derived overwhelmingly from extracavernous colluvial sources. The contribution of the insoluble residue produced by intracavernous corrosion is chiefly restricted to a few highly angular chert particles.

Several generations of colluvium are evident near Sterkfontein; these range from the pebbly, skeletal mantles in areas where a balance exists between erosion and deposition, to less frequent, deeper, pedogenetically differentiated profiles in relict areas immune from rapid denudation. The –2-mm fractions usually display fairly similar ranges of properties; these seem related rather to a geomorphic environment of episodic incision along the Bloubaank valley and to local depositional conditions than to climate and weathering, the imprint of which should rather be sought in fluctuations in patterns and rate of sedimentation within the protected environments of cave depositories.

The characteristics of the Sterkfontein Formation are most readily explained in terms of the reworking of source materials akin to the present, widespread, thin, pebbly colluvium. This is a dark reddish-brown (5 YR 3/4), sandy loam (slightly clayey silty sand), containing abundant weathered chert pebbles and cobbles, and seldom exceeding 50 cm thick. The ϕ mean averages 4.20. The material is poorly to very poorly sorted and is leptokurtic with a small range of skewness, both negative and

positive. Crystalline minerals are quartz, goethite, kaolinite, sericite and feldspar. Roundness and sphericity are 0.3 and 0.5, and many particles show pyrolusite staining.

Sedimentary facies of the various units of the Sterkfontein Formation can be related to the extracavernous colluvium in terms of a limited suite of transportation and depositional processes. Except in the case of Member 3, where enrichment in fines, possibly due to the presence of an aeolian component, has resulted in pronounced positive skewness, the colluvial source materials have suffered removal of fines to various degrees. This can be attributed to the flushing action of successive sheetfloods through the cave opening, which tended to transport fine elements into more distant depositories within the cavernous dolomite, increasing negative skewness except where further transportation was obstructed (for example, in areas where debris cones impinged on the walls of the cave).

Fine sedimentary fractions are more abundant in the lower members, due to the greater degree of illuviation with increasing distance from the cave opening. An exception is Member 6, the proximity of which to the cave opening would be expected to favour a depletion in fines through flushing and eluvial effects. The relatively high proportion of fines encountered in this member suggests that the colluvial source materials have undergone limited transportation into a restricted depository with enrichment in fine elements.

The extensive hiatuses in clastic deposition within the cave, sometimes associated with calcite accretion, indicate cycles with reduced influx of extracavernous sediments. In some instances such episodes were marked by intra-cavernous scouring and the production of erosional unconformities.

Under the present climatic and pedogenetic regime in the area the very coarse fraction of the colluvium consists exclusively of partially weathered chert fragments derived from the chert-rich dolomite. Material of this type is present in all members, and has sometimes undergone post-depositional weathering. Only in bed B of Member 4 does weathered dolomite form a significant component of the very coarse colluvial fraction. The contemporary occurrence of weathered dolomite in colluvial mantles in South Africa is restricted to arid environments.

Solution cavities and discrimination between members

Mention must be made of the unconsolidated fillings of solution cavities which were formed within the soluble surface of the Sterkfontein Formation, chiefly of Member 5, after the cessation of sedimentation. These attain depths in excess of 5 m and contain material produced by decalcification and *in situ* weathering of the surrounding sediments. A clear transition exists between the overlying recent colluvium and these residual materials. The latter sometimes contain bone. The weathering residue from Member 5, which occurs within these cavities, is sedimentologically similar to its parent material, but somewhat better sorted, indicating enrichment in fines, either through *in situ* weathering and comminution of particles, or through illuviation from the overlying colluvium. Individual particles are also more rounded than those from Member 5, and pyrolusite staining is widespread.

Sedimentological analysis of samples from the various lithostratigraphic units of the Sterkfontein Formation reveals that successive members are distinctive in terms of the standard deviation of at least one of the associated quartile and moment measures.

Comparison with Swartkrans Formation

The stratigraphy of the nearby Swartkrans hominid site has been re-appraised recently^{14,15}, and the Swartkrans Formation has been defined. Comparison of the Sterkfontein and Swartkrans Formations shows that, although similarities exist, there is no obvious lithostratigraphic overlap between their various members and beds. The sediments of the Swartkrans Formation are distinctly more positively skewed and are generally more

poorly sorted than those of the Sterkfontein Formation. While the accretion of Member 1 of the Swartkrans Formation in a gravitative debris cone is analogous to that of Members 4 and 5 of the Sterkfontein Formation, the subsequent large-scale corrosion of this accumulation, and the filling of the voids thus created with younger sediments, followed, in turn, by a second cycle of channel erosion and deposition, is not apparent at Sterkfontein. The preponderance of sediments derived from deep argillaceous colluvium in both Members 1 and 2 of the Swartkrans Formation¹⁴ likewise has no parallel at Sterkfontein.

The faunal assemblage from the Type Site at Sterkfontein (labelled StS) is overwhelmingly derived from Member 4 of the Sterkfontein Formation; it is very different from that of the Extension Site, and has no equivalent at Swartkrans¹⁶. Previous evaluations of the assemblage from the Sterkfontein Extension Site¹⁷⁻²⁰ have assumed that the entire West Pit assemblage (labelled SE) belonged to one stratigraphic unit. My studies reported here, confirmed independently by C. K. Brain, reveal that some specimens from this assemblage have attached matrix belonging to Members 4 and 6 of the Sterkfontein Formation, even though Member 5 is the most widespread stratigraphic unit exposed in the West Pit. In view of this discovery, previous evaluations of these faunas with respect to chronology will have to be revised. A much needed comparison of the faunas of Member 1 of the Swartkrans Formation and Member 5 of the Sterkfontein Formation awaits a further study of the revised assemblage from the latter unit (to be undertaken shortly by E. S. Vrba).

The fauna from Member 2 of the Swartkrans Formation¹⁵ includes elements from both the main mass of this unit and the later channel fillings. Vrba's¹⁶ earlier and later subdivisions of the SKb bovid fauna can be equated with these two units. Vrba^{16,21} has concluded that the later SKb fauna corresponds to her later Sterkfontein category, which is likely to belong to Member 6 of the Sterkfontein Formation.

These comparative faunal data are not sufficiently precise to permit postulation of time-equivalence between the respective stratigraphic units.

Correlation with previous terminology and hominid finds

It may prove useful for readers familiar with the published stratigraphic terminology of previous workers at Sterkfontein to have a cross-reference to the terminology used here (Table 1). At the same time, an indication is also provided of the relative position of the excavations carried out to date and the known of likely provenance of certain of the hominid remains.

From the above correlation it will be clear that excavations so far have sampled only the uppermost 30% of the stratigraphic thickness of the Sterkfontein Formation. No excavations have been carried out in Members 1-3.

I have not considered biostratigraphic or chronostratigraphic aspects of the Sterkfontein Formation here; these, together with the environmental implications of the data, will be discussed elsewhere.

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Neuronal properties underlying processing of visual information in the barnacle

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Generation of a transient, amplified response to the dimming of light in the visual system of the barnacle involves two synaptic stages. It is accomplished primarily by decrementally conducting neurones that are similar to bipolar cells of the vertebrate retina.

THIS work is concerned with the fate of visual information as it is processed by the first few cells of the simple visual system of the barnacle. The specific problems addressed here have been raised by observations on the vertebrate retina. As information is passed from the receptors to bipolar cells and from them to the retinal ganglion cells, the steady responses that encode light intensity are essentially lost, whereas responses to changes in intensity are amplified. How the synaptic and membrane properties of decrementally conducting retinal cells accomplish this process of differentiation, which enables ganglion cells to perceive changes over a wide intensity range, is not understood (see, for example, refs 1–4). Like their counterparts in the vertebrate retina, the first few cells of the visual pathway of the barnacle are specialised to extract information about changes in intensity.

Voltage changes induced by light in the dendrites of the photoreceptor cells of the barnacle median eye spread decrementally along the cells' axons to their terminals in the supra-oesophageal ganglion^{5,6}. Light depolarises the photoreceptors and dimming causes them to repolarise. In cells which we conclude to be second- and third-order, however, dimming causes a transient depolarisation. This depolarisation generates a burst of action potentials in the third-order cells. These impulses are conducted through the circum-oesophageal connectives to the ventral ganglion to influence neurones that mediate the shadow-induced withdrawal of the animal into its shell^{7,8}. The present study is of the synaptic connections and membrane properties of the neurones that process the receptor signal to generate an 'off-response' over a wide range of light intensities.

Identification of second- and third-order ganglion cells

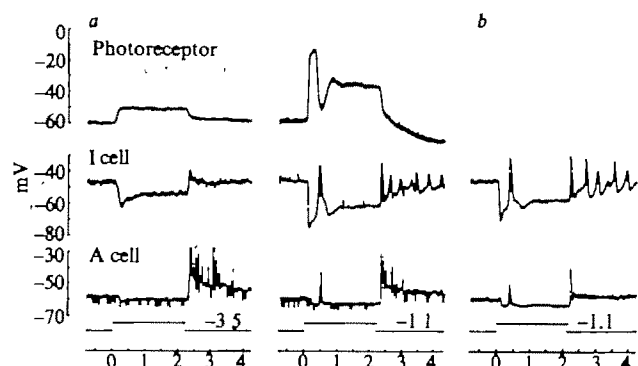
Many of the neurones of the supra-oesophageal ganglion respond to the dimming of light, and are probably involved in the complex defensive response of the animal to a shadow^{9,10}. Most cells do not respond to the onset of a pulse of light but only to its termination. Certain small cells near the photoreceptor terminals, however, respond to the onset as well as to the termination of light^{11,12}; at light onset these cells hyperpolarise, and at the termination of light they depolarise transiently. As these 'biphasically responding cells'¹³ sense both the onset and

the offset of light, they must precede other cells in the visual pathway. Indeed, their hyperpolarising and depolarising responses, as well as the photoreceptor responses, are spared when sodium action potentials are eliminated by tetrodotoxin (TTX), abolishing all activity in other ganglion cells.

We have investigated further the properties of biphasically responding cells and have found that they can be divided unambiguously into two types on the basis of their characteristic responses to light, their dark resting potentials, and their ability to generate sodium- or calcium-dependent action potentials. So far we have found only one cell of each type on each side of the ganglion.

The responses of a photoreceptor and of each type of ganglion cell to pulses of dim and bright light are presented in Fig. 1. The response of one type, the 'inverting cell' (I cell), is essentially a mirror-image of that of the receptor. Evidence that the I cell is a second-order neurone, in other words directly postsynaptic to the photoreceptors, will be presented below. The I cell displays a low resting potential in the dark (mean -45 mV; range -30 to -50 mV; $n=9$), a marked hyperpolarisation at the onset of light, and a depolarisation or oscillations at the termination of the light pulse. The second type of biphasically responding cell

Fig. 1 Responses to light of a photoreceptor, an I cell and an A cell. *a*, Responses in normal saline to dim (left-hand traces) and moderately bright (right-hand traces) pulses of light of 2 s duration. I cell record is atypical in that most I cells generate off-responses, even following bright lights, which consist of a single depolarisation overshooting the dark resting potential, rather than of the multiple oscillations shown here. *b*, Responses of the same cells shown in *a* when the ganglion was perfused with TTX 10^{-6} g ml⁻¹. The photoreceptor impulse was lost after changing solutions. The recordings from the photoreceptor and I cell were made simultaneously; the A cell responses are from another experiment. Light intensities (log mW cm⁻²) are indicated to the right of the shutter monitor trace (lower trace).



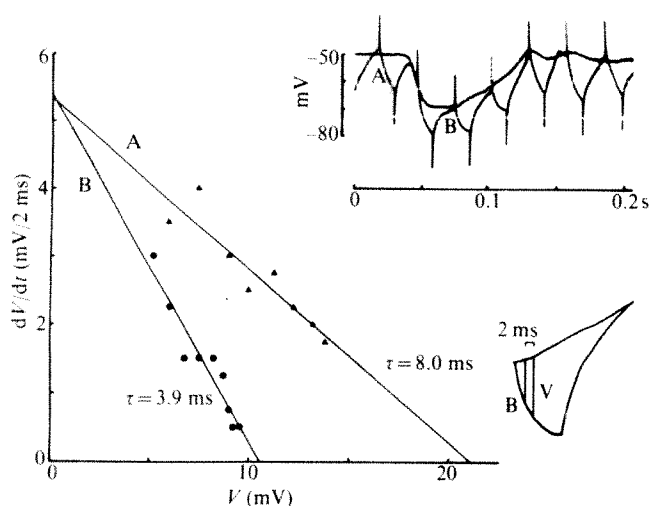


Fig. 2 Conductance of the I cell increases during its response to light. The experiment is illustrated at the top right. Two oscilloscope sweeps are superimposed. The voltage response to a flash of light was recorded by one sweep; short pulses of current of constant magnitude were superimposed on the flash response on the other sweep. The changes in voltage evoked by these current pulses were larger and had a longer time constant in the dark (for example, A) than during the light response (for example, B). To obtain a value for the time constant of the membrane during light and dark independent of bridge balance, a phase-plane plot was made relating the rate of change of the voltages (dV/dt) in pulses A and B to the voltage (V). Ordinate, dV/dt , measured as the difference between voltage measurements at 2 ms intervals during the rising phase of the hyperpolarising pulse (diagram, lower right). Abscissa, V , membrane potential of cell (see diagram). The exponential increase in the negative voltage in time is described by $V = V_{\infty}(1 - \exp(-t/\tau))$ where V_{∞} is the voltage developed at the end of a long current pulse and τ is the time constant. To relate V and dV/dt , one can take the time derivative of the above expression: $dV/dt = (V_{\infty}/\tau) \exp(-t/\tau)$. These two expressions can each be solved for $V_{\infty} \exp(-t/\tau)$ and equated. Therefore $dV/dt = -V/\tau + V_{\infty}/\tau$. This equation describes the lines drawn through the data points on the phase-plane plot by linear regression. From the slopes of each line the time constants of rise of pulses A and B were calculated using the above equation and are shown. As the time constant is inversely proportional to the conductance of the cell, the conductance is increased by a factor of about two.

has been called an 'amplifying cell' (A cell), as the most conspicuous feature of its response is a large depolarisation after termination of illumination even when the concurrent voltage changes in the receptors and I cells are small. The A cell has a resting potential (mean -64 mV; range -55 to -70 mV; $n = 17$) more typical of ganglion cells and the photoreceptor itself; it gives only a small hyperpolarisation to light, but a large depolarisation which sets up action potentials after termination of illumination. Previously published work has dealt with neurones with the properties of A cells¹¹⁻¹⁴ and has shown that impulses arising in these cells at the termination of a light pulse are conducted along the cells' axons in the contralateral connectives.

The hyperpolarising and depolarising responses to light of both I cells and A cells are preserved in TTX (Fig. 1b). In TTX, the A cell is no longer able to generate action potentials, and its background synaptic activity ceases.

One simple way to account for the observations presented in Fig. 1a and b is that the I and A cells are the second- and third-order neurones in the visual pathway. When depolarised by light, the photoreceptors release inhibitory transmitter on to the I cells, causing them to hyperpolarise. In turn, the I cells, when released from inhibition by dimming or by dark, depolarise and release excitatory transmitter on to the third cell in the sequence, the A cell. This scheme is consistent with the

observation that I cells in the dark rest at a relatively depolarised membrane potential which could cause them to release transmitter constantly. Such a circuit has been proposed by Ozawa *et al.*¹⁴ as one likely way of accounting for their observations on cells with the properties of A cells.

The proposed scheme implies that I cells would have a greater conductance in the light, when they are bombarded with inhibitory transmitter, than in the dark. A cells would display just the opposite properties: a greater conductance in the dark when they are bombarded with excitatory transmitter than in the light. We have found that the conductance of the I cell is indeed increased in response to light (Fig. 2). The conductance of cells with properties of A cells has been measured^{13,14} and was found to be greater in the dark than in the light. The direction of the conductance changes in these two biphasically responding cell types supports the hypothesis that the receptor neurone releases inhibitory transmitter in the light and the I cell releases excitatory transmitter in the dark.

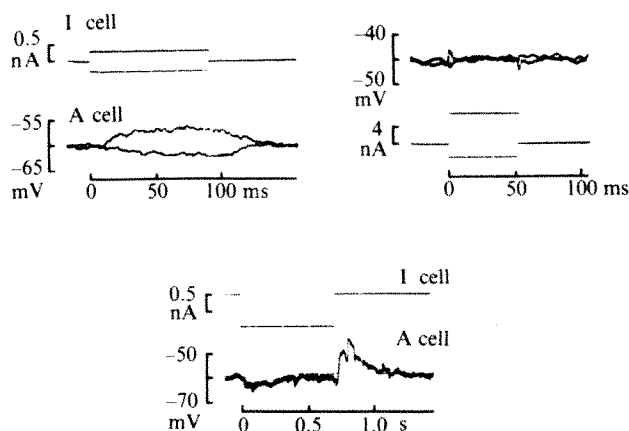
Recordings made simultaneously from an I cell and an A cell show that the I cell is presynaptic to the A cell. Injection of current in the dark into the I cell in either the depolarising or hyperpolarising direction causes a steady potential change of the same sign in the A cell (Fig. 3a). This result would be expected if polarisation of the I cell were increasing or decreasing a steady release of excitatory transmitter from this neurone. The long latency between I cell polarisation and A cell response suggests that transmission at this synapse is chemically mediated. Injection of current into the A cell causes no change in the I cell membrane potential (Fig. 3b). Thus, the I cell is presynaptic but not postsynaptic to the A cell. Hyperpolarising currents injected into the I cell to mimic the effect of light can cause a depolarisation in the A cell at the cessation of the current pulse (Fig. 3c), much like the off-response to light.

Functional membrane properties of I cells and A cells

Both I cells and A cells can display regenerative activity associated with repolarising potential changes in the photoreceptor (Fig. 1a and b). The nature of this regenerative activity is decidedly different, however, in the I cell and A cell.

The activity that one sees in the I cell ranges from oscillations to small, slow (about 20 ms to peak) regenerative events with noticeable inflections on the rising phases. This regenerative activity is insensitive to TTX (Fig. 1b). Hyperpolarising current pulses injected into the cell soma evoke oscillations at the break

Fig. 3 Depolarisation or hyperpolarisation of an I cell causes potential changes of the same sign in the contralateral A cell (upper sets of traces). Polarisation of the A cell does not affect the I cell (lower set of traces). The magnitude of the injected current was measured by recording the command potential which drove the current pump in the d.c. preamplifier. The experiment was carried out in the dark.



of the pulse (Fig. 4). Distinct action potentials of greater amplitude can be evoked if the preparation is bathed in 100 mM tetraethylammonium ion (TEA). This compound generally blocks voltage-sensitive potassium channels, thereby reducing outward potassium currents and allowing inward currents to generate larger voltage changes than in normal saline. The amplitudes and rates of rise of the action potentials generated in the presence of TEA are decreased when calcium is replaced by magnesium. When calcium is replaced by barium in the presence of TTX, an action potential can be generated even in the absence of TEA. Thus, the regenerative activity recorded from the soma of an I cell is due to voltage-sensitive calcium channels.

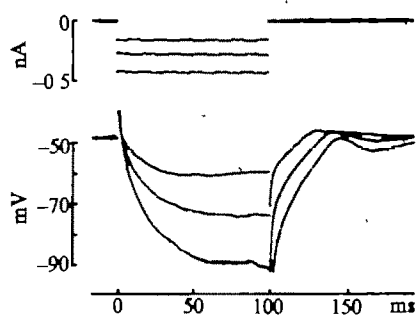


Fig. 4 Oscillations evoked in the I cell at the break of a hyperpolarising current pulse. Three pulses of increasing amplitude have been superimposed. Upper traces: current monitor, current recorded as in Fig. 3. Lower traces: membrane potential.

The impulses set up in the A cell are, on the other hand, abolished by TTX. A cell impulses are always distinct action potentials rather than oscillations, they are conducted along the cell's axon in the circum-oesophageal connective, and they have the fast rise time (1–2 ms) typical of sodium-dependent action potentials.

Information transfer at the first two synaptic stages

Both the peak and plateau of the I cell response (Figs 1 and 5a and b) grow over a range similar to that of the receptor response (ref. 5, Fig. 4). Thus, information concerning the intensity of maintained light is transferred from the receptor to the I cell. Figure 5a shows that the membrane potential of the I cell is a function of light intensity over a range of about 5 log units. The slope of this relationship varies widely among different I cells for reasons as yet unknown.

Figure 6 shows a simultaneous recording made from a photoreceptor and an I cell. The steep slope of the relationship between intensity and plateau response in this I cell shows that receptor potentials as small as 1 mV cause a response in the postsynaptic cell (Fig. 6a). For voltage changes in the receptor ranging from 1–20 mV, the membrane potential in the I cell was roughly proportional to the membrane potential of the photoreceptor (Fig. 6b).

In contrast to the plateau response of the I cell (Fig. 5a), that of the A cell saturates at about $10^{-3.6}$ mW cm $^{-2}$ (Fig. 5b). According to our hypothesis, the A cell plateau is the resting potential of the cell in the absence of synaptic input; at light intensity dimmer than $10^{-3.6}$ mW cm $^{-2}$, the A cell is depolarised by excitatory transmitter released from the I cell. In brighter lights the I cell seems to be hyperpolarised enough so that it stops releasing transmitter.

The essence of the A cell response to a light pulse is its depolarising off-transient. When the ganglion is bathed in TTX, this depolarisation no longer elicits action potentials and its amplitude can be measured. The amplitude of this off-transient is a function of both the intensity and the duration of the

preceding light pulse (Fig. 5d). The off-depolarisation increases with light intensity for dim lights and saturates at moderately bright light. As the amplitude of this saturated response, and the intensity at which it saturates, depends on the duration of the preceding light as well as on its intensity, information concerning absolute light intensity is not preserved at the A cell stage.

Discussion

The physiological observations presented above suggest that visual information passes serially from the photoreceptors of the median eye to decrementally conducting I cells and from them to A cells. The A cells transmit information about dimming to the ventral ganglion.

At the first integrative stage, the synapse between the photoreceptors and the I cells, the light response is inverted by an inhibitory synapse. I cells respond to changes of membrane potential in photoreceptors as small as 1 mV from dark resting potential (–60 mV). Thus, release of transmitter from the receptor must take place at membrane potentials more negative than those in squid presynaptic terminal¹⁵ and even takes place at the dark resting potential (B. Schnapp, personal communication). Release of transmitter at the well characterised synapses in the squid, lamprey and the hatchetfish nervous systems, is not detectable until the presynaptic cell has been depolarised by roughly 20 mV to about –45 mV (refs 15–17). The input–output relationship of the receptor synapse has therefore been shifted so that release, and by inference calcium entry¹⁸, occurs at more negative voltages.

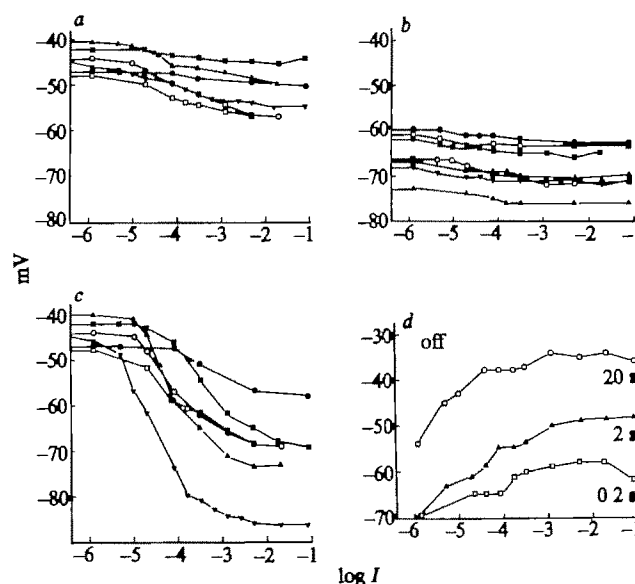


Fig. 5 Responses to light of I cells (a and c) and A cells (b and d). Plateau hyperpolarisations measured in six I cells (a) and seven A cells (b) just before the end of a 2-s light pulse and plotted as a function of light intensity. c, Peak hyperpolarisations of the I cells plotted as a function of light intensity. d, amplitudes of off-responses in an A cell (▼ in b) as a function of light intensity for light pulses of three different durations as indicated. The experiment illustrated in d was carried out in the presence of TTX.

Responses of the I cell to light also resemble those of bipolar cells of the vertebrate retina. In both, the responses are graded, the onset and termination of light evoke potential changes from dark rest in opposite directions to one another, and neither cell type normally generates all-or-none action potentials. Bipolar cells have been reported to exhibit oscillations in certain conditions^{19,20} which have the appearance of small action potentials but are graded in amplitude. These oscillations in the bipolar cell are similar to those sometimes seen in the I cell and might similarly be due to voltage-sensitive calcium channels.

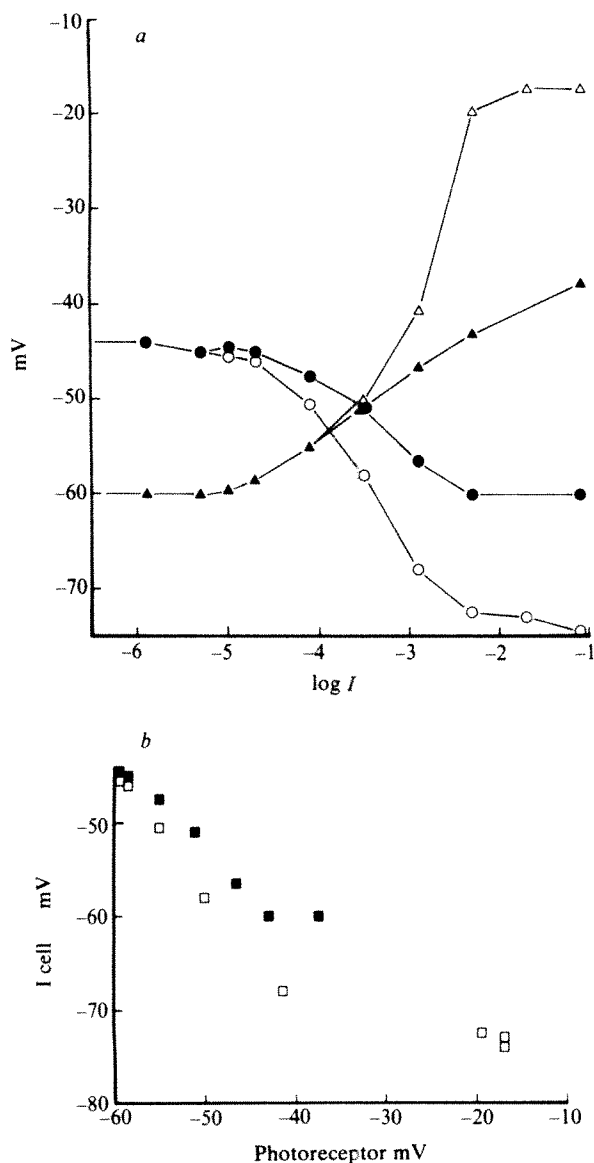


Fig. 6 Relationship between membrane potentials of an I cell and a photoreceptor recorded simultaneously. *a*, Peak (Δ) and plateau ($\bullet$) hyperpolarisations in the photoreceptor and peak ($\square$) and plateau ($\blacksquare$) hyperpolarisations in the I cell as functions of light intensity. Plateaus were measured at the end of a 2-s light pulse. *b*, Plot of the membrane potential changes induced by light in the photoreceptor against changes in the I cell. The data from *a* are replotted showing relationship of peak ($\square$) and plateau ($\blacksquare$) measurements of light responses from the two cells. Dark resting potentials were -44 mV in the I cell and -60 mV in the photoreceptor. The steepness of the relationship is not significant, as it depends on the exact location of the electrode along the length of the photoreceptor axon (6.6 mm from the ganglion in this experiment). This I cell is represented as $\circ$ in Fig. 5*a* and *c*.

The responses of I cells also share features of second-order cells in insect visual systems. Intracellular recordings have been made from second-order neurones (laminar type I neurones²¹) in lateral eyes of several insects (locust²² and fly²³⁻²⁵) and in the median eye of the dragonfly²⁶. Light responses of second-order cells in these insects resemble light responses in the I cell in that they have a large, transient, initial hyperpolarisation which decays to a plateau.

It is not clear whether second-order neurones in insects generate action potentials. As action potentials have been seen occasionally in these insect cells²⁶, the absence of impulses from intracellular recordings is usually attributed to microelectrode damage^{23,26}. On one occasion, however, we have recorded

action potentials from I cells in normal saline which resembled those recorded from second-order cells of the dragonfly ocellus but which were insensitive to TTX. Perhaps the impulses recorded occasionally in insect second-order cells are calcium-dependent.

At the second integrative stage in the barnacle, the synapse from the I cell to the A cell, the intensity range over which the responses of A cells are graded is narrowed and the off-response is amplified (Fig. 5*b*). When I cells are released from a hyperpolarisation caused by light or by current, regenerative activity is seen. We have shown that this activity reflects the activation of voltage-sensitive calcium channels, and it is reasonable to assume that these channels are at least in part those associated with the release of transmitter. The onset of dark, then, causes depolarisation of the I cell, the activation of voltage-sensitive calcium channels, and the release of transmitter which excites the A cell. The release properties of this synapse are such that the graded portion of the light response is for the most part not transmitted to the third-order cell. The oscillation occurring in the I cell when the light goes off, on the other hand, is transmitted and amplified. The large depolarisation occurring in the A cell at the termination of light generates a burst of TTX-sensitive action potentials.

We have suggested that the I cell constantly releases transmitter on to the A cell in the dark. Like Ozawa *et al.*¹⁴ we have observed that in the dark the A cell membrane potential undergoes greater fluctuations than it does in the light. Ozawa *et al.*¹⁴ suggest that this increased noise arises from fluctuations in the continuous release of transmitter on to the cell in the dark and that in the light transmitter release stops.

We have observed excitatory and inhibitory synaptic potentials on both the I cell and the A cell in various conditions. The role of these inputs is not understood; they might well contribute to the light responses in I cells and A cells. It seems unlikely, however, that a third type of biphasically responding cell would be interposed between the receptor and I cell, or between the I cell and A cell, as in our many impalements of small cells in many preparations we have found only these two types of cells which respond when light is turned on. Final confirmation of our postulated scheme must come from the demonstration of direct contact between the cells with the electron microscope.

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Evidence for splicing of interrupted immunoglobulin variable and constant region sequences in nuclear RNA

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Evidence is presented that the mouse light-chain coding sequence is interrupted in a 27S nuclear RNA species, whereas the sequence is continuous in both a 13S nuclear RNA and in cytoplasmic mRNA. The discontinuity of coding regions in the 27S nuclear RNA parallels the situation found in myeloma DNA and indicates, therefore, that the removal of interruptions in the V and C regions occurs at the level of nuclear RNA.

IMMUNOGLOBULIN (Ig) polypeptides consist of variable (V) and constant (C) regions associated in a single molecule. This molecule is synthesised, by the antibody-producing cells, from a single mRNA molecule containing both V and C regions¹. Recent restriction enzyme mapping data^{2,3} on mouse κ class light (L) chains have shown that the germ-line DNA carries the V and C genes separated by an unknown stretch of DNA. Differentiation of antibody-producing cells seems to result in DNA rearrangement such that the V and C genes are moved closer together in the genome^{2,3}. It has been shown, however, that such a rearrangement does not result in a fully contiguous stretch of DNA carrying V and C_h genes in a linear array³ but rather the active κ L-chain gene of myeloma cells contains an interruption sequence at or near the junction of the V and C regions: A similar interruption has also been observed in a mouse myeloma λ L-chain⁴. Therefore, the transcription unit encoding the V and C genes includes material which is not represented in the final mRNA.

Recently, data have been obtained from several other systems, showing the occurrence of discontinuous genes. These appear in adenovirus and SV40 (refs 5–11), ribosomal cistrons^{12–16}, globin genes^{17,18} and ovalbumin genes^{19,20}. One possible route by which this extra material may be removed is during the processing of the nuclear RNA (nRNA) precursors of mRNA. Thus, the entire Ig gene (including the sequence of the interruption) can be transcribed into a large nuclear RNA precursor followed by a process of ligation to form contiguous V and C regions. Alternatively, it is possible that the V and C regions could be transcribed into separate nRNA molecules from which the V and C genes are joined. A completely different type of process might involve the RNA polymerase 'jumping' across the interruption sequence (thereby effectively ignoring it) and generating contiguous sequences in RNA from discontinuous sequences in DNA. We have investigated the arrangement of V- and C- region sequences in the nuclear RNA of MOPC 21 cells and conclude that transcription of the interrupted Ig gene produces a nRNA molecule or molecules in which an interruption occurs at or near the V–C junction. Joining of this V–C interruption, therefore, apparently occurs at the level of nuclear RNA to generate a 13S nuclear RNA molecule with continuous V and C regions.

Characteristics of a 27S nRNA containing L-chain sequences

RNA was prepared from detergent-washed nuclei of mouse MOPC 21 cells, and fractionated on a sucrose gradient following

denaturation in dimethyl sulphoxide (DMSO). An aliquot of each gradient fraction was hybridised with ³²P-labelled L-chain complementary DNA (cDNA) and the single strand-specific S₁ nuclease-resistant radioactivity determined by trichloroacetic acid (TCA) precipitation (Fig. 1). The main peak of hybridisation observed was around 13S but significant hybridisation was found in regions up to about 30S (a minor but reproducible peak

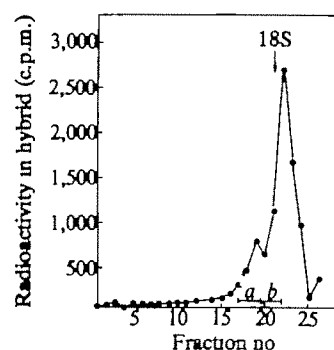


Fig. 1 Hybridisation of L-chain cDNA to fractionated mouse myeloma nuclear RNA. Nuclear RNA was prepared from mouse myeloma P3K cells (MOPC 21)²¹ by a modification of a previous procedure²². 10⁶ cells were lysed in 20 ml 0.5% NP40, 0.24 M sucrose, 10 mM Tris-HCl pH 7.5, 10 mM MgCl₂, 10 mM KCl. The nuclei were pelleted and suspended in 1 ml 0.12 M sodium phosphate pH 6.8, and lysed with 100 ml 8 M urea, 0.24 M sodium phosphate pH 6.8, 1 mM EDTA, 0.5% SDS²³. 10 g hydroxyapatite (Biorad) were added and stirred vigorously for 10 min. The hydroxyapatite plus the DNA was removed by centrifugation (5 min at 1,000g) and the supernatant dialysed exhaustively against 0.25 M NH₄Ac, 1 mM EDTA, 0.5% SDS. RNA was recovered by ethanol precipitation and dissolved in 10 ml 7 M urea, 0.35 M NaCl, 0.5% SDS and 1 mM EDTA. This solution was extracted twice with an equal volume of buffer-equilibrated phenol-chloroform (1:1 v/v) and ethanol precipitated. The recovered RNA was washed twice with 3 M NaAc pH 6.0 (ref. 24) to remove residual DNA, reprecipitated from 100 mM NaAc pH 6.0, and finally dissolved in 1 mM EDTA, 70% DMSO. The solution was heated for 2 min at 70 °C, rapidly diluted with 3 vols 0.5% SDS, 100 mM NaCl, 1 mM EDTA and 10 mM Tris-HCl pH 7.5, and layered on 15–40% sucrose gradients (in 100 mM NaCl, 0.5% SDS, 10 mM Tris-HCl pH 7.5, 1 mM EDTA). Centrifugation was for 5 h at 38,000 r.p.m. in an MSE 6 × 14 ml rotor at 25 °C. The gradients were fractionated into 0.5-ml fractions and 100-μl aliquots were ethanol precipitated with 10 μg *Escherichia coli* DNA plus 2,500 c.p.m. unfractionated ³²P-L-chain cDNA. The recovered precipitate was dissolved in 20 μl 0.3 M NaCl, 10 mM Tris-HCl pH 7.5, 0.5% SDS and 1 mM EDTA and incubated at 70 °C for 16 h. The hybridised cDNA was estimated by TCA precipitation following S₁ nuclease digestion as previously described². The cDNA used in this study was made with MOPC 21 L-chain mRNA and T₂-G₃-T as primer. This primer initiates at the equivalent of residue 205 just within the C region^{25–27}, so cDNA molecules begin with C_h sequences and proceed into the V region. The V+_h cDNA used in Fig. 2 consisted of a cDNA band eluted from an acrylamide gel; this band was about 700 bases long and represents the maximum length transcript which can be obtained from the T₂-G₃-T priming site. Sedimentation is right to left. Pools a and b were selected for resedimentation.

of hybridisation being observed at around 27S). Evidently, therefore, the steady-state nRNA of MOPC 21 cells consists mainly of a species showing a similar sedimentation value to 13S cytoplasmic mRNA. A second species (around 27S) seems to be present in the nRNA but in rather low proportions: this RNA will be approximately two to three times the size of the L-chain mRNA and presumably represents a precursor of the L-chain mRNA. This conclusion is consistent with a recent publication²⁸ which showed that, although a 40S species is the probable initial transcript of the L-chain gene, a 13S species was the predominant L-chain-specific nuclear RNA present, with a 24S minor species also being observed.

We have investigated the spatial arrangement of the V and C regions in these nuclear RNA species with an assay previously used to demonstrate the presence of an interruption in the κ L-chain gene³. This assay consists of electrophoretic determination of the size of ³²P-cDNA, protected from S₁ nuclease digestion, after hybrid formation with DNA or RNA. The rationale of this approach is that cDNA containing V- and C-region sequences would be completely resistant to S₁ nuclease after hybridisation with RNA or DNA in which the V- and C-region sequences are continuous. On the other hand, this cDNA would be only partially resistant to S₁ nuclease when hybridised to RNA or DNA in which interruptions of V and C regions occur. In a previous study³ we found that V + C cDNA (that is, cDNA of about 560 bases in length, made as described in Fig. 1 legend, extending over both C and V regions) was not fully protected from S₁ nuclease when hybridised with myeloma DNA prepared from cells making the specific L-chain. Instead,

we observed a major band of protected cDNA (about 290 bases in length) corresponding approximately to the C region. These results indicated the presence of an interruption at or near the junction of V and C_κ regions and also indicated that the V_κ gene may possess interruption(s), as we failed to detect a cDNA fragment consistent with the expected V-gene length³. This assay provides a convenient method to study nuclear RNA precursors, as the S₁ nuclease protection of V + C cDNA by nuclear RNA will exhibit half protection of the type observed with myeloma DNA (DNA-like protection) if the nRNA precursor contains interruptions of the V and C regions or full protection if the nRNA precursor contains continuous V and C regions.

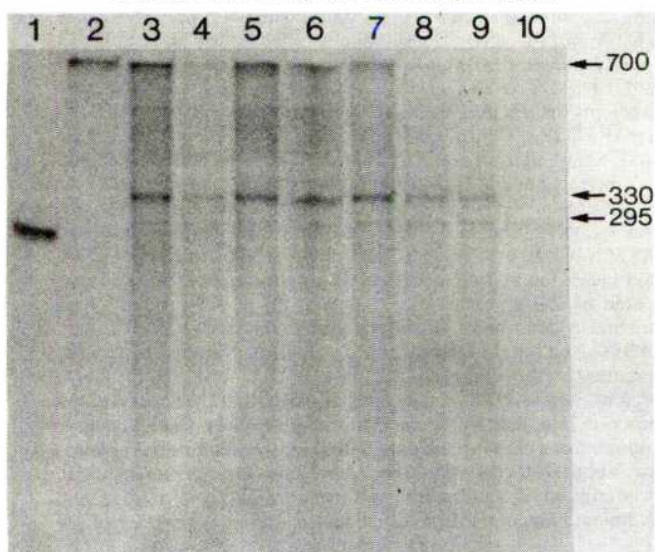
Figure 2 shows the pattern of S₁ nuclease protection of V + C cDNA (700 bases) obtained by hybridisation with various nRNA gradient fractions (from Fig. 1). The pattern of cDNA protected from S₁ nuclease by hybridisation with cytoplasmic mRNA shows two major bands (slot 3): one band of intact cDNA 700 bases in length (complete protection) and a further band of partial protection around 330 bases. This latter material does not occur when purified 13S L-chain mRNA is used³ and possibly results from a specific processing of the RNA. This protection pattern of the cDNA was also found in the higher molecular weight region of the fractionated nRNA but, in addition to this, a further component was observed, with a peak of sedimentation around 27S. This component consisted of an RNA species capable of only partially protecting the 700-base V + C cDNA (after hybridisation) to yield a protected cDNA fragment around 295 bases. This partially protected cDNA fragment is similar to the 'half protection' which we previously observed with experiments on myeloma DNA (that is, DNA-like protection)³ and presumably reflects interruption sequences present in the 27S RNA which are absent from the 13S mRNA. Therefore, in the nuclear RNA there are apparently two separate components, the first having a sedimentation value around 27S, possessing a DNA-like arrangement of V and C regions, and the second with a value around 13S and partly possessing continuous V and C regions (that is, mRNA-like arrangement of V and C regions). Thus, the primary transcripts of the κ -chain gene seem to possess the interruption which occurs at or near the V- and C-region junction.

Confirmation of interrupted V and C regions in the L-chain 27S nRNA

The sedimentation time used for the nRNA described in Figs 1 and 2 was short so as to display any putative nRNA molecules larger than 27S. The result was that the resolution of the 27S and 13S species was poor, and considerable overlap of the two patterns of S₁ nuclease protection was displayed by the various gradient fractions in Fig. 2. To increase the resolution of these molecules and to characterise the 27S molecule further, the two regions of the gradient shown in Fig. 1 (a and b) were pooled and resedimented after further denaturation in DMSO. The hybridisation profiles of L-chain cDNA with these various fractions are shown in Fig. 3. Both pools a and b, as expected, showed hybridisation with L-chain cDNA at a position consistent with a 13S RNA species, and in addition showed peaks of hybridisation at around 27S (pool a) and 20S (pool b). No hybridisation with L-chain cDNA was observed if the nRNA pools were boiled in alkali before resedimentation. Therefore, the material sedimenting at 27S is indeed a high molecular weight nRNA species containing L-chain sequences. The only 'steady-state' precursor molecule which we observe is the 27S molecule, whereas the nature of the 13S nRNA is more uncertain. This RNA is probably a true nuclear species but could be partly contamination of nuclear RNA by 13S cytoplasmic mRNA.

The pattern of S₁ nuclease protection of cDNA after hybridisation to the resedimented nRNA pools (a₁, b₁, and a₂ + b₂, Fig. 3) clearly shows the difference between the 27S and the 13S nRNA, as cross-contamination of the 27S RNA with 13S RNA

Fig. 2 Pattern of S₁ nuclease protection of L-chain cDNA after hybridisation with fractionated mouse myeloma nRNA. 100- μ l aliquots of fractions 17–23 (from Fig. 1) plus 100- μ l aliquots of cytoplasmic mRNA were hybridised to V + C cDNA as in Fig. 1. Following hybridisation, the nucleic acids were precipitated with ethanol and recovered by centrifugation. The pellets were washed with 70% aqueous alcohol, dried *in vacuo* and dissolved in 20 μ l 0.28 M NaCl, 30 mM NaAc pH 4.5, and 4.5 mM ZnSO₄ (ref. 29). S₁ nuclease digestion was carried out for 1 h at 37 °C. Following digestion, the mixtures were diluted fivefold with 0.2 M sodium acetate pH 5.0, 5 mM EDTA and 20 μ g ml⁻¹ *E. coli* tRNA, and ethanol precipitated. The recovered pellet was washed once with 70% alcohol, dried and dissolved in 20 μ l 95% formamide by boiling for 3 min. The denatured samples were electrophoresed in 4% polyacrylamide gel in 8 M urea and the bands visualised by exposure to prefogged Fuji X-ray film at -70 °C using a fast tungstate intensifying screen. Slot 1 is a marker cDNA fraction of ~280 bases; slot 2 is the unhybridised 700-base V + C cDNA; slot 3 is the S₁ protection pattern of cDNA after hybridisation to cytoplasmic mRNA; slots 4–10 show the S₁ protection pattern of cDNA after hybridisation to gradient fractions 23–17 (that is, fraction 23 is the low molecular weight region of the gradient and fraction 17 is the high molecular weight region).



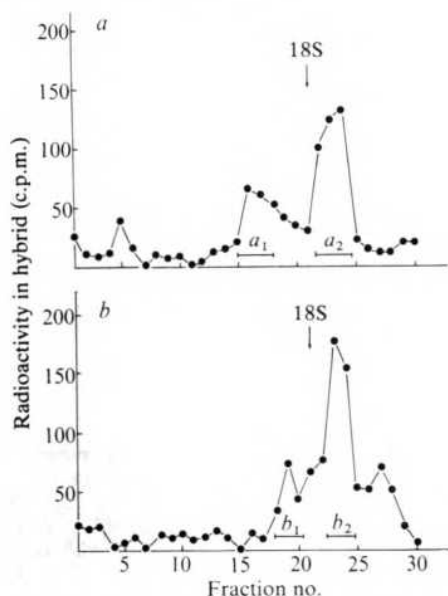


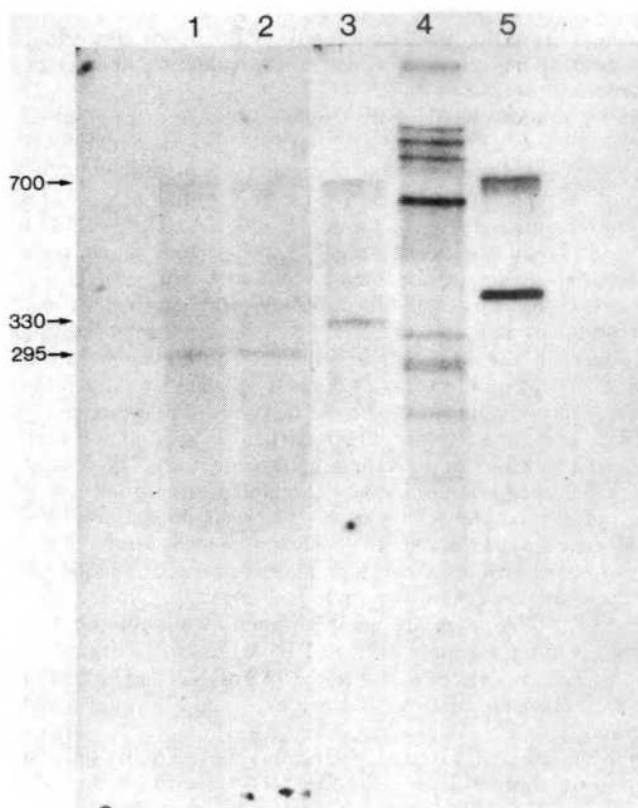
Fig. 3 Hybridisation profiles of cDNA with resedimented nRNA pools. The regions of *a* and *b* shown in Fig. 1 were pooled and the RNA recovered by ethanol precipitation. The RNA was denatured in DMSO and resedimented as in Fig. 1. Aliquots were taken and hybridised with unfractionated L-chain cDNA as in Fig. 1 followed by estimation of S_1 nuclease-resistant cDNA (TCA-precipitable counts). Sedimentation is right to left. The upper panel shows the hybridisation pattern of pool *a* and the lower panel that of pool *b*. The regions a_1 , b_1 and $a_2 + b_2$ were selected for further study.

was limited after the resedimentation. In addition, this experiment should be viewed in relation to the ability of purified 13S mRNA to protect *V + C* cDNA fully from S_1 nuclease³. Figure 4 shows the size of cDNA protected from S_1 nuclease after hybridisation of *V + C* cDNA (700 bases) to the nRNA pools. Both the 27S (a_1) and 20S (b_1) pools exhibit DNA-like protection patterns in that the major protected component of the *V + C* cDNA (initially 700 bases) was about 295 bases long. (This size estimate is based on a comparison with radioactive *Hae*III digestion fragments of Φ X174 which were run in a parallel slot of the gel.) The principal feature of these protection patterns is, therefore, the ability of the 27S nRNA to yield a protected cDNA fragment of about 295 bases in length which parallels the situation with myeloma DNA. The result, therefore, demonstrates that a sequence occurs in the initial transcripts, of the κ gene, which is absent from the mRNA. A small amount of intact *V + C* cDNA is observed after hybridisation with the 27S nRNA: this material may result from contamination with mRNA-like sequences and/or incomplete digestion of the cDNA. The 13S pool ($a_2 + b_2$) shows a different pattern of protection, yielding two bands of approximately equal quantities (700 and 330 nucleotides) of which the fully protected *V + C* 700-base component reflects the fully integrated state of at least part of the 13S nRNA. There is no evidence that the 295-base component results from hybridisation of cDNA to 13S nRNA, indicating that splicing events have occurred to eliminate this interruption at around the *V-C* junction. The origin of the 330 component is at present obscure, but as we were unable to detect such a component in purified L-chain mRNA³, we feel that the component is derived from nuclear RNA. We are, therefore, investigating the possibility that this material is a consequence of the nuclear RNA splicing which may involve, for example, putative interruptions in the V_κ -gene sequence.

To provide independent confirmatory evidence for the presence of interruption sequences in the 27S nRNA, we have compared the nucleotide sequences of the 13S mRNA and 27S nRNA in the area of the *V-C* junction (Fig. 5). To facilitate this comparison, cDNA was made complementary only to the C_κ region by using T_2 - G_3 -T primer in a reverse transcription using limiting concentrations of dCTP (0.05 μ M). The cDNA which

results from this reaction is mainly 100–200 bases in length. As the T_2 - G_3 -T initiates just within the *C* region^{25–27}, material less than 298 residues in length is complementary only to C_κ sequences. Thus, when the C_κ cDNA is hybridised to the C_κ sequences of either mRNA or nRNA and re-extended using the hybrid RNA as a new template, the sequence of the extended cDNA will be governed by the sequence of the new template. By incorporating the dideoxynucleotides as chain terminators in the re-extension transcription³⁰, we can generate a series of sequence-specific stopping points which can be visualised by fractionation of the resultant cDNA on acrylamide gels³¹. Figure 5 shows this sequence comparison using mRNA or 27S nRNA as template and dideoxy-TTP as the chain terminator. Clearly, the cDNA has increased in size as a result of annealing and re-extension on the RNAs (slots 2 and 3) compared to the starting cDNA population (slot 1). By comparison with the sequence of the mouse C_κ region²⁷, we can approximately position the bands on the gel with respect to amino acid residues in the protein. For reference in Fig. 5 an arrow has been marked at the T residue present in the codon corresponding to amino acid 110 (Asp) which is close to the *V-C* junction as defined by amino acid sequence data. Therefore, the sequence approximately up to this residue is C_κ -region sequence. Clearly, the cDNA band patterns resulting from extension on the mRNA and on the 27S nuclear RNA are identical up to around this residue, demonstrating that the C_κ sequences of these RNA species are identical. In addition, this result shows the lack of interruptions in the C_κ sequence of the nuclear RNA (at least

Fig. 4 The pattern of S_1 nuclease protection of L-chain cDNA by resedimented nRNA pools. Pools a_1 , b_1 and $a_2 + b_2$ (from Fig. 3) were ethanol precipitated, hybridised with *V + C* cDNA, digested with S_1 nuclease and fractionated on a polyacrylamide gel to visualise the protected cDNA fractions as in Fig. 2. The sizes of the various cDNA bands were calibrated using denatured nick-translated *Hae*III-digested Φ X174 (slot 4) and a 362-base fragment of *Xenopus* 5S DNA (slot 5). Mixed with the latter fragment in slot 5 was a sample of the *V + C* cDNA (700 bases). The other slots show the S_1 protection pattern of pool a_1 (slot 1), pool b_1 (slot 2) and pools $a_2 + b_2$ (slot 3). Slots 1 plus 2 and 3 to 5 have been exposed for different times for maximum display.



between the equivalent of amino acids 110 and 205). However, the cDNA band patterns obtained using the 27S nRNA clearly begins to differ from that obtained with the cytoplasmic mRNA at a position which approximates to the V-C junction. This difference of band pattern therefore reflects a difference of base sequence of the two RNA species after this area of the sequence. Thus, the result confirms unequivocally the presence of a sequence in the 27S nRNA which is absent from the mRNA. This interruption sequence is presumably adjacent to the C_κ region, which means that the MOPC 21 V region is not adjacent to the C_κ in this 27S nRNA. Localisation of the precise point of sequence divergence in the two RNA species must await actual sequence data on the 27S nRNA itself.

Two steps in the integration of V and C genes

The results described indicate that two RNA populations can be isolated from the same nuclei of MOPC 21 cells; one of these nRNA species exhibits a sequence arrangement of V and C genes similar to that in mRNA (that is, continuous V and C regions), and the other nRNA (27S) exhibits the interrupted arrangement of V and C regions which occurs in the DNA itself. The most likely scheme of events would, therefore, seem to be that the 27S species is a precursor of the 13S nRNA and that the final removal of interruptions from V and C regions occurs in processing events of nRNA. Evidence in favour of the 27S molecule as a precursor of L-chain mRNA comes from pulse-labelling experiments²⁸. In these experiments a shortlived 40S nRNA was found to be the initial transcript of the *Ig* gene in MOPC 21 cells. This 40S molecule was shown to be the precursor of a 24S molecule, and in turn the 24S RNA was found to be the precursor of the 13S nRNA. This 24S nRNA species is presumably equivalent to the 27S nRNA molecule discussed here and thus, this 27S molecule would be the precursor of the 13S nRNA and cytoplasmic mRNA.

It is impossible to say whether V and C regions are on the same molecule or whether the 27S fraction contains one molecule with the C region and another molecule with the V region. One piece of indirect evidence suggests that the V and C regions are on the same molecule. This evidence comes from experiments with myeloma/spleen cell hybrids³² in which, for example, there is no mixing of the C_H sequences and the V_H sequences, from the two parental cells, in the hybrid line. Final resolution of this point however, must await further characterisation of the 27S nRNA.

Integration of the V and C genes has commonly been supposed to occur at the level of DNA. We now know that this is only partially true and are able to present a two-step model for integration of V and C_κ genes (Fig. 6). The first step of the integration seems to be at the level of DNA. The inherited germ-line DNA carries separate V and C_κ genes which, by a process of DNA rearrangement, seem to be brought closer together in the DNA of the antibody-producing cell^{2,3}. An interruption remains in the DNA at or near the V and C_κ junction even after this rearrangement³. These events are the classical 'integration' events as defined by genetics. This relocation of the *Ig* genes would be the mechanism by which a cell becomes committed to produce a particular V gene and may also be responsible for activating the *Ig* gene itself. The second step of the integration process occurs after transcription of the gene and involves elimination of the interruption sequences of V and C genes in the nuclear RNA. The initial transcript(s) of the active *Ig* gene includes the V region, the interruption sequence and the C region. The discontinuous V and C_κ regions of the precursor nRNA(s) are presumably joined by a looping out of the intervening sequence followed by ligation of V and C_κ regions. This process gives rise to the 13S nRNA and in turn to active cytoplasmic mRNA where the V and C_κ regions are continuous.

In this discussion, we have specifically referred to the splicing of the interruption at or near the V-C junction by nRNA processing. This processing scheme will also probably be

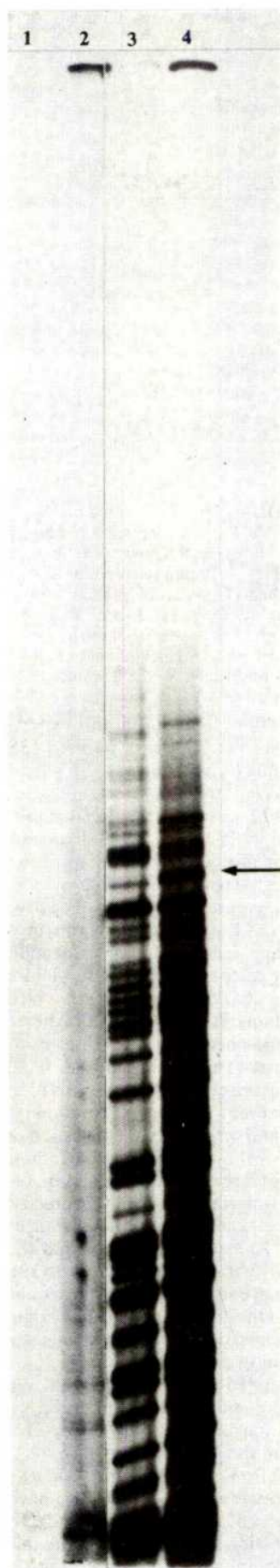


Fig. 5 Comparison of the sequences near the V-C junction of 13S mRNA and 27S nRNA. ^{32} P-cDNA was prepared with reverse transcriptase using MOPC 21 κ mRNA as template and T₂-G₃-T as primer^{26,27}, with 0.05 μ M dCTP and 10 μ M dGTP, dTTP plus 4 μ M ^{32} P-dATP. After incubation for 30 min at 37 °C the reaction mix was boiled for 5 min with 0.5 M NaOH neutralised with 1 M NaH₂PO₄, and the cDNA purified by chromatography on Sephadex G-100. The cDNA was annealed with either κ mRNA or 27S nRNA in 100 mM NaCl at 60 °C for 1 h. The cDNA was re-extended in the RNA:cDNA hybrid with reverse transcriptase in fresh reactions containing 100 μ M dATP, dCTP, dTTP and dGTP plus 10 μ M dideoxy-TTP³⁰ for 30 min at 37 °C and fractionated on 8% polyacrylamide gel in 8 M urea³¹. Slot 1, ^{32} P- Φ X174 digested with *Hae*III; slot 2, ^{32} P-cDNA starting material (not re-extended); slot 3, ^{32} P-cDNA re-extended on 13S messenger RNA; slot 4, ^{32} P-cDNA re-extended on 27S nuclear RNA. The arrows represent the position of a T residue in the codon equivalent to amino acid residue 110.

responsible for removing other interruptions which may occur in *Ig* genes. It has recently been shown, for example, that a germ-line V_{λ} gene possesses a short interruption in its linear sequence³³, and it is possible that V_{λ} genes possess insertions, as we were unable to detect a complete *V*-region size fragment in S_1 nuclease protection experiments with either MOPC 21 DNA³ or the 27S nRNA described here. It is also likely that nuclear RNA processing will be a general process for removal of the interruptions which have been found in other eukaryotic genes and in viral systems. Indeed, it has recently been shown that a globin nuclear RNA precursor possesses an interruption sequence which is absent from the mRNA³⁴.

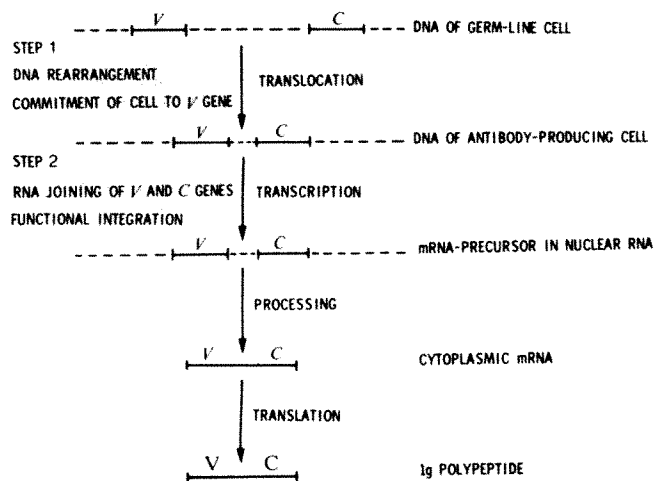


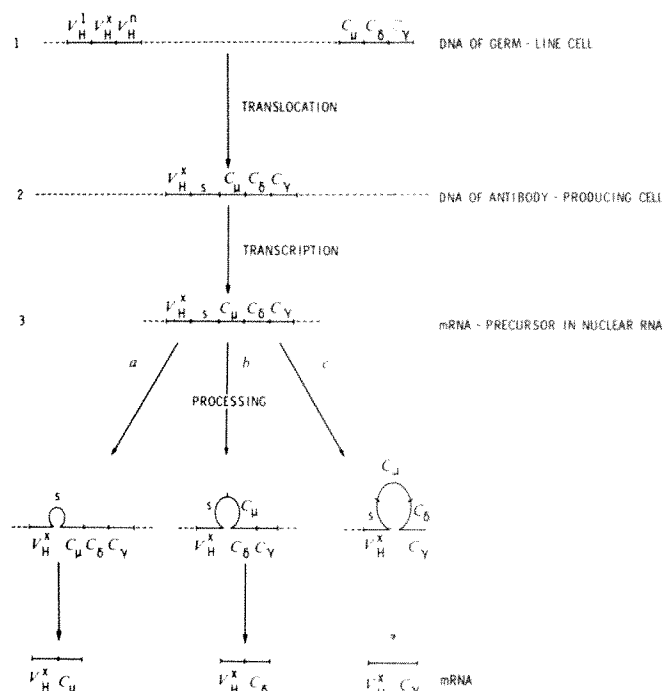
Fig. 6 Two-step model for integration of variable and constant region genes. Step 1, the inherited germ-line DNA carries separate *V* and *C* genes. These genes are brought closer together in the antibody-producing cell, but an interruption at or near the *V*-*C* junction remains in this DNA. This rearrangement or translocation of DNA is the classical 'integration' event and it is the event which allows the individual antibody-producing cell to become committed to a *V* gene. Step 2, transcription of the activated gene occurs to produce a nuclear RNA precursor which encompasses the *V* and *C* regions plus the interruption sequence(s). Functional integration of the *V* and *C* regions occurs during the processing of the precursor molecule, presumably by a looping out of the interruption sequence(s) followed by ligation of *V* and *C* regions. The sequence arrangement of the mRNA is thus achieved and from this the Ig polypeptide is made. It is predicted that any further insertions (such as may exist in the *V* region) will also be eliminated by nuclear RNA splicing.

Hypothetical scheme for Ig heavy-chain gene expression

The joining of *V* and *C* genes in the nuclear RNA may well explain some of the problematical features of Ig heavy(H)-chain gene expression. During the immune response there is a shift from IgM to IgG production, which seems to be the result of a switch of H-chain class within single clones^{35,36}. It has been shown that a single myeloma patient produces an identical V_H sequence in IgM and IgG^{37,38}, and other studies have shown that single cells apparently produce identical V_H regions in association with C_{μ} and C_{δ} (refs 39, 40). Thus, a cell becomes committed to a heavy-chain *V* gene and this same *V* gene can, at various times, associate with C_{μ} , C_{γ} and C_{δ} . It is also possible that a further association with C_{α} can occur, as an identical V_H sequence has been found in a myeloma patient producing IgG and IgA⁴¹. There does not seem to be a recommitment of the antibody specificity of a given cell, and previous models have envisaged either successional insertion of a V_H gene to the different C_H genes³⁷ or simultaneous insertion of copies of the V_H gene with the C_H genes⁴². A further model may now be proposed in which the processing of heterogeneous nuclear RNA is the site of association of the 'committed' V_H gene with the various C_H genes (Fig. 7). Thus, a single V_H gene is selected

from the V_H -gene pool and DNA rearrangement occurs to bring the V_H gene to the proximity of the C_H -gene array. Primary transcription of the active H-chain gene unit may now occur to produce a giant nuclear RNA precursor containing the V_H region and the various C_H -gene classes. The cell can now produce from one nuclear RNA precursor species the same V_H region associated with any of the C_H classes by processes of looping out intervening sequences, ligation of the V_H and C_H regions, followed by degradation of the unwanted RNA. A single cell, therefore, using such an nRNA precursor, could produce simultaneously or sequentially one, two or three mRNA species containing the same V_H sequence with the different C_H sequences. As shown in Fig. 7, this is a minimal model involving only the C_{μ} , C_{δ} and C_{γ} genes: there are some data suggesting the involvement of C_{α} but little evidence for involvement of C_{ϵ} . The minimal model can, however, easily be adapted to include these C_H genes if necessary by simply hypothesising their inclusion in the giant nRNA molecule. A general

Fig. 7 Hypothetical scheme for expression of a single heavy-chain V_H gene with multiple C_H genes. (1) The V_H -gene pool is depicted as a set of genes linked to the C_H -gene array. The order of the C_H genes is hypothetical and for simplification no subclasses of the C_H genes are shown. A single V_H gene (V_H^x) is selected for expression and DNA rearrangement occurs to bring the V_H^x and C_H genes closer together. A *V*-*C* interruption sequence may or may not exist between V_H^x and C_H (depicted as S). This process of rearrangement will commit the cell to a V_H gene and may also activate the H-chain transcription unit. (2) Transcription of the H-chain unit produces a giant nRNA precursor encompassing the V_H^x -gene *V*-*C* interruption sequence (if this occurs) and the C_H genes. (3) Intranuclear processing of this giant nRNA molecule can give rise to any combination of V_H^x and C_H in mRNA. Thus, (3a) $V_H^x C_{\mu}$ mRNA can be generated by looping out of the *V*-*C* interruption sequence, ligation of V_H^x and C_{μ} followed by degradation of the remaining RNA; (3b) $V_H^x C_{\delta}$ mRNA by looping out sequence 'S' plus the C_{μ} sequence followed by ligation of V_H^x with C_{δ} ; (3c) $V_H^x C_{\gamma}$ mRNA can be made from the same nRNA precursor by analogous processing events. A single cell can therefore simultaneously make one, two or three mRNA species with the same V_H gene and different C_H genes. 'Spacer' sequences must occur between the C_H genes to allow specificity of processing: these putative sequences would, of course, be eliminated by the nRNA processing. The model shown is a minimal model, as it excludes C_{α} and C_{ϵ} . It is possible that C_{α} (and C_{ϵ}) could also be involved in the pathway, in which case they can readily be accommodated by adapting the hypothetical giant nRNA precursor to include one or both of these C_H genes.



feature of the model is that it makes very clear predictions which can be tested experimentally.

In the way described, a single event (that is, the event responsible for V_H -gene commitment) is required for both activation of the heavy-chain gene transcriptions and multiple expression of the same V_H gene with any of the C_H genes at the level of mRNA. How is the required specificity of RNA joining likely to be achieved with a 'multicistronic' nRNA precursor of this type? Presumably, this joining is mediated through an enzyme or enzymes which can be either specific for the different genes or nonspecific, relying on RNA folding properties to achieve specificity. If specific enzymes are used it means that a large array of different species must exist, whereas nonspecific enzymes require only intrinsic properties of the gene sequence itself for correct joining. There are as yet no clues about the processes of RNA joining but doubtless, *in vitro* systems will be useful in exploring this aspect of RNA joining.

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letters to nature

X-ray emission from the companion to V861Sco

THE Copernicus satellite has observed V861Sco (Hr6283, Spectrum; $B0.5$ Ia, $V = 6.2$, $B - V = +0.28$, $U - V = -0.72$) as part of a programme devoted to the study of mass transfer in binary star systems. The programme described here uses the Princeton UV telescope¹ to make detailed measurements of absorption lines in the UV spectra of such stars. X-ray measurements are made simultaneously with a co-aligned proportional counter².

Significant signals from the X-ray detector were obtained during the first observation from 24 to 26 April 1978. The flux recorded, averaged over 1.2-h intervals, is shown in Fig. 1. At the end of the observation on 26 April a significant fall in intensity was observed. The hypothesis that this fall in intensity was caused by an eclipse of the X-ray source, was tested by a second observation which began on 27 May 1978. Results of this second observation are shown in Fig. 2. The flux was observed to decline at a time consistent with the known 7.8-d period⁷ of V861Sco. The low flux state lasts at least 1.3 d and the decline to this level occurred over a 0.7-d period.

There is no evidence of regular modulation (as is common with neutron star sources) greater than 15% peak to mean flux in the range 3-40 min.

The X-ray spectrum, observed when the signal was at maximum intensity, is shown in Fig. 3. Appreciable photoelectric absorption is present. The column density is equivalent to

$(4 \pm 1) \times 10^{23}$ H atoms assuming normal cosmic abundances and a thermal spectrum with a temperature equivalent to 11 ± 4 keV. The interstellar column of both atomic and molecular hydrogen to this region is $(2.3 \pm 1.0) \times 10^{21}$ atoms³. There is, therefore, considerable photoelectric absorption intrinsic to the source.

The Princeton guidance system maintains the pointing accuracy of both instruments to better than 0.1 arc s throughout the observing period. However, the beam width of the X-ray detector is 2.5° by 3.5° , full width at half maximum (FWHM), so the field has been examined for catalogued sources. No known sources were within the field with the exception of 4U1702-42 which was 2.8° from the centre for the V861Sco observations. 4U1702-42 is catalogued⁴ at 30 Uhuru flux units and would only contribute 3% or less than 1 OAO count in 62.9 s (the Crab nebula gives 375 OAO counts in this integration period). The signals observed by the Copernicus instrument thus indicate the presence of a new source which has been designated OAO 1653-40 (ref. 5).

The variable star V861Sco was shown by Walker to be a spectroscopic binary with a 7.8-d period and a mass function of 0.487 solar masses ($M_\odot$). A collapsed object was suggested as a possibility for the unseen companion⁷. Although Walker assumed the primary to have a mass 10 times that of the Sun ($10 M_\odot$), more reasonable estimates would be 20-30 $M_\odot$ implying a mass of 7-11 $M_\odot$ for the secondary, the actual value depending on the inclination of the system. This range is above the limit for a neutron star indicating that the compact secondary is a black hole.

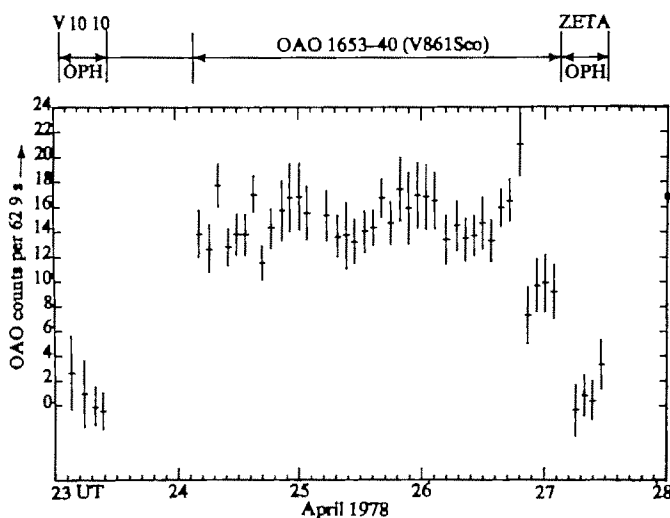


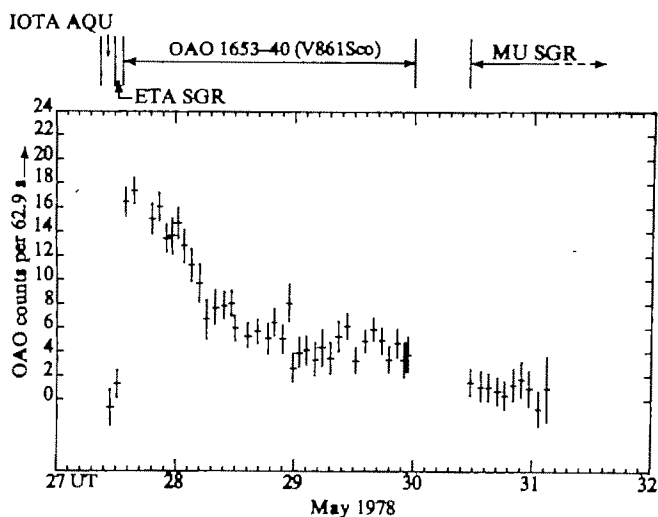
Fig. 1 First observation of V861Sco. The significant drop in the X-ray signal can be seen at the end of the observation. Times of observation of other target stars (used for background determination) are also indicated.

The X-ray observations support the identification of OAO 1653-40 with V861Sco. The two reductions in signal are separated by exactly four cycles of the binary star orbit and this is the strongest evidence for the suggested identification. A new orbital ephemeris is required to relate the phasing of the X-ray and optical data. UV observations obtained in three spectral regions ($\lambda\lambda$ 1110-1130, 1167-1185 and 1230-1247) during the X-ray eclipses indicate a stellar spectrum similar to that of κ Ori. Broad, strong, violet-displaced (by about -700 to -800 km s $^{-1}$) absorption components are seen in C III λ 1176, N V λ 1238 and λ 1242. Weak Nv emission may also be present. These observations are, however, insufficient to relate the phases of the X-ray source and V861Sco.

The observed X-ray luminosity (3.8-8.8 keV and assuming a Crab-like spectrum) is given by $4 \times 10^{34} R^2$ erg s $^{-1}$ where R is the distance to the source in kpc. V861Sco is a member of the association Sco OB1 (ref. 8) and using the distance (1.95 kpc) to this cluster we obtain an X-ray luminosity of 1.5×10^{35} erg s $^{-1}$. The emission outside this energy band and electron scattering near the source increase the estimate of luminosity, possibly by several orders of magnitude.

When the X-ray source is in eclipse there is a significant residual signal at approximately 0.25 the non-eclipsed value.

Fig. 2 Second observation of V861Sco in which the expected eclipse is observed after four cycles of the binary star had passed since the first observation.



Further observations are needed to clarify the nature of this; for example, it may be a contaminating point source or arise from a possible supernova remnant in this association⁹.

The observed properties of OAO 1653-40 are so similar to those described for 4U1702-42 (ref. 4), that one might question whether the position of the latter is in error. Preliminary analysis of more recent data, obtained in July 1978, indicates that the intensity of the X-ray source has reduced considerably, implying that the source is transient within the sensitivity limits

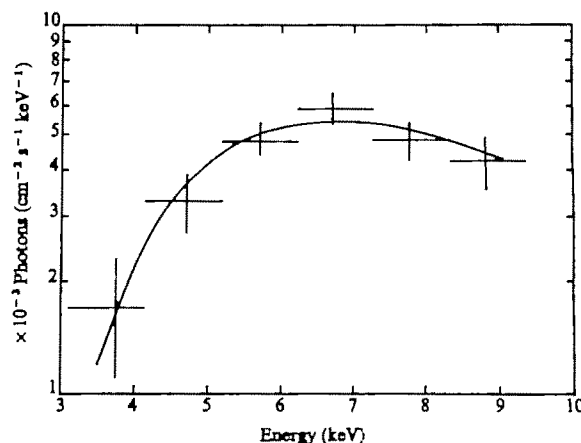


Fig. 3 The observed X-ray spectrum when the signal is at maximum intensity. A thermal spectrum has been fitted to the data but other spectral forms are not precluded. Significant photoelectric absorption is evident. Solid curve, thermal spectrum:

$$P(E) = \frac{(0.120 \pm 0.005)}{E} \exp\left(\frac{-E}{11 \pm 4}\right) \exp\left[(4 \pm 1) \times 10^{23} \sigma_{\text{BG}}\right]$$

(σ_{BG} are the Brown and Gould¹⁰ cross-sections for interstellar absorption).

of the Copernicus detector. If the association of the X-ray source with V861 is correct then either the known variable mass flow⁶ has caused further obscuration or has reduced accretion which has diminished the X-ray output.

We thank N. E. White for providing the constraints to regular pulsations.

Note added in proof: Clarification of the optical ephemeris and additional X-ray measurements suggest the observed decreases in flux are caused by the 'eclipse' of the X-ray source by a dense gas stream in the system. A true eclipse of the X-ray source by the primary star has recently been observed with Copernicus in further observations.

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COS B observation of high-energy γ radiation from 3C273

THE discovery of a high-energy γ -ray source at $\alpha(1950)=12^{\text{h}} 29^{\text{m}} \pm 6^{\text{m}}$, $\delta(1950)=+3^{\circ} \pm 1.5^{\circ}$ is reported here. Arguments are given for the identification with 3C273. If this identification is correct, the γ -ray luminosity of 3C273 in the energy range 50–500 MeV is $2 \times 10^{46} \text{ erg s}^{-1}$ for $H_0=60 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

The majority of the reported γ -ray sources are galactic, as can be clearly established from their latitude distribution¹. The lack of unambiguous identification (with the exception of CG185–5 with PSR0531+21 and of CG263–2 with PSR0833–45) leaves a wide range of speculations as to the classification of the sources and to the nature of the emission mechanisms. The outstanding signature of these sources is the fact that their γ -ray luminosity ($>50 \text{ MeV}$) exceeds at least the radio and X-ray luminosities. Besides the continuing search for galactic γ -ray sources, some observations of the ESA γ -ray observatory COS B have been devoted to extragalactic targets². The region of the sky centred on $\alpha=12^{\text{h}} 24^{\text{m}}$, $\delta=+7^{\circ}$ with an effective field of view of about 20° radius was observed from 24 May to 24 June 1976. This observation was particularly aimed at a sensitive search for high-energy γ -ray emission from M87 and 3C273.

The data have been analysed using a cross-correlation method: the frequency distribution of arrival directions of γ rays was correlated with the distribution expected for a point source as determined by calibration and confirmed by flight data on the strong source PSR0833–45 (ref. 2). The resulting correlation map follows the distribution expected for random data, apart from one clear maximum, which has a probability of 10^{-5} of being caused by random fluctuations at any position in the map. This excess follows the distribution expected for a point source located within a 90% probability error box defined by $\alpha(1950)=12^{\text{h}} 29^{\text{m}} \pm 6^{\text{m}}$, $\delta(1950)=+3^{\circ} \pm 1.5^{\circ}$. Spectral information was obtained by analysing the data in two energy ranges. In each case a significant excess within the same error box was found. The derived characteristics of this source (designated CG291+65) are summarised in Table 1. The errors quoted include the estimated systematic errors.

Table 1 Characteristics of CG291+65

Position	$\alpha(1950)=12^{\text{h}} 29^{\text{m}} \pm 6^{\text{m}}$	$\delta(1950)=+3^{\circ} \pm 1.5^{\circ}$
Intensity	50–150 MeV	$(1.2 \pm 0.4) \times 10^{-6} \text{ photons cm}^{-2} \text{ s}^{-1}$
	150–500 MeV	$(0.3 \pm 0.1) \times 10^{-6} \text{ photons cm}^{-2} \text{ s}^{-1}$
Energy flux	50–500 MeV	$3 \times 10^{-10} \text{ erg cm}^{-2} \text{ s}^{-1}$

Of the two specific targets of this observation, M87 is about 8° outside the error box, and is therefore clearly not the source of the detected γ rays. On the other hand, 3C273 lies well within the error box. Despite the large size of this error box, the following compelling arguments for an identification with 3C273, the second nearest of the powerful QSOs ($z=0.158$), can be made. The probability of finding a known high-galactic-latitude X-ray source by chance within a $3^{\circ} \times 3^{\circ}$ error box is about 2%. However, the X-ray source 4U1226+02 ($\equiv 2A1225+022$) lies within the error box of CG291+65. This X-ray source has been positively identified with 3C273 (refs 3, 4). Also, it is stressed that the probability of finding a chance coincidence of the error box with a specific object, that is 3C273, is $<1\%$.

If this identification is correct the observed intensities at γ -ray energies can be compared with those at other wavelengths, as shown by the spectrum in Fig. 1. This shows that a major portion of the energy radiated by 3C273 is in the form of γ rays. Its γ -ray luminosity in the range 50–500 MeV is $\sim 2 \times 10^{46} \text{ erg s}^{-1}$ for $H_0=60 \text{ km s}^{-1} \text{ Mpc}^{-1}$.

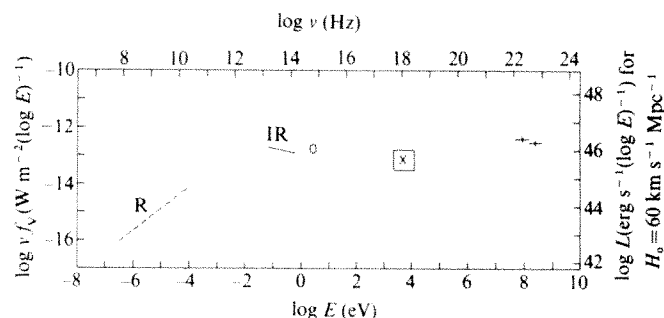


Fig. 1 Spectrum of 3C273 from radio to γ -ray energies. The intensity scale is given in units of power per decade. The radio (R), optical (O) and X-ray (X) data are those given in a compilation by Apparaio *et al.*⁵. The IR data are from Rieke and Low⁶.

This has important consequences for the understanding of QSOs and warrants extensive efforts to further strengthen the identification. Repeated observations of 3C273 may yield signs of time variability, similar to those clearly established at other wavelengths, with amplitudes which tend to increase with increasing energy⁵. A second observation by COS B is planned for June/July 1978. Somewhat less directly, the identification could be strengthened by an extensive search for γ -ray emission of other QSOs. In this context, note that the newly discovered and nearest QSO, associated with 2S0241+622, lies within the error box of CG135+1 (ref. 5).

The intriguing fact that all γ -ray sources detected by COS B, including pulsars, unidentified galactic sources and possibly extragalactic sources, have a peak luminosity at γ -ray energies, may indicate some similarity in the emission process, or may reflect a selection caused by the limited dynamic range of detected γ -ray fluxes.

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Infrared observations of the most luminous quasar

The QSO Q 0420–388 is one of many discovered by Osmer and Smith in their objective prism survey of the southern sky, and one of ~ 10 now known with $z > 3$. Its brightness and large redshift indicate a luminosity at rest wavelength $1,475 \text{ Å}$ somewhat larger than any other measured. The source is particularly noteworthy because of the steepness of its observed visual spectrum over the range $3,600\text{--}6,800 \text{ Å}$, where $F_v \sim$

$\nu^{-2.1}$; this suggests that appreciably more power might be radiated in the infrared (IR). The near IR photometric observations reported here of Q 0420-388 ($z = 3.12$) show that it is 13.5 ± 0.2 mag at $2.2 \mu\text{m}$. This observation and the optical observations of Osmer and Smith¹ indicate a bolometric luminosity $\geq 5 \times 10^{14} L_{\odot}$ for $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and $q_0 = 0.1$ confirming Q 0420-388 as the most luminous quasar for cosmologies with small q_0 .

Table 1 gives accurate positions for the quasar and a nearby star, as measured from the ESO B survey plates. These positions are derived from those of the five nearest SAO stars (194936, 194939, 194974, 194977, 194979) including the effects of the proper motions of these stars.

Using the 1.5 m telescope at CTIO and the InSb photometer of the Center for Astrophysics, we observed Q 0420-388 at $2.2 \mu\text{m}$ ($1.36 \times 10^{14} \text{ Hz}$) on 27 July 1977 and 30 July 1977, obtaining consistent results whose weighted mean is $K = 13.5 \pm 0.2$ mag, corresponding to a $2.2 \mu\text{m}$ flux of $2.5 \pm 0.5 \text{ mJy}$. We used a $35''$ diameter beam, and a beam separation of $96''$ in declination. The star in Table 1 is the only one visible on the Whiteoak extension of the Red Palomar Sky Survey that might interfere with our measurement of the quasar. This star is $34''$ SE, well outside our beam, and a separate measurement of its brightness gave $K = 12.9 \pm 0.7$ mag; the star is too faint and too far separated for scattered light from it to affect our measurement of the QSO.

The flux distribution $\lambda F_{\lambda} = \nu F_{\nu}$ is given in Fig. 1. Osmer and Smith¹ give a flux of 0.6 mJy at $6,077 \text{ \AA}$ (rest wavelength $1,475 \text{ \AA}$). Combined with our result, this gives $F_{\nu} \sim \nu^{-1.1 \pm 0.2}$ for the range $0.6-2.2 \mu\text{m}$. This spectral index is consistent with the range of values quoted by Osmer and Smith for the $0.36-0.68 \mu\text{m}$ region, and it may be that the spectral index of the quasar is -1.1 ± 0.2 over the entire $0.36-2.2 \mu\text{m}$ range. The high galactic latitude of the source, $|b| \sim 45^\circ$, suggests that the galactic extinction is low. Thus, a change in the apparent spectral index of the source would reflect its intrinsic behaviour. Q 0420-388 is not listed in the Parkes catalogue², so its flux at $2,700 \text{ MHz}$ cannot be more than 220 mJy . This result and our $2.2 \mu\text{m}$ measurement indicate that the spectral index between the radio and near IR is > -0.4 . From their observations of high redshift QSOs in the Molonglo survey, Baldwin *et al.*³ infer that there is a preponderance of such flat spectrum sources among high z objects.

We have estimated the bolometric flux using $\nu F_{\nu} \sim \nu^{-1}$ for $\lambda < 6,077 \text{ \AA}$, $\nu F_{\nu} \sim \nu^{-0.1}$ for $0.61 \mu\text{m} < \lambda < 2.2 \mu\text{m}$, and $\nu F_{\nu} \sim \nu$

Table 1 Precise positions

Object	$\alpha(1950)$				$\delta(1950)$			
	h	m	s	s	°	'	"	"
Q 0420-388	04	20	30.07	± 0.05	-38	51	50.4	± 0.6
Star SE of QSO	04	20	31.95	± 0.05	-38	52	16.9	± 0.6

for $\lambda < 2.2 \mu\text{m}$. This gives $F_{\text{bol}} = (1.05 \pm 0.2) \times 10^{-14} \text{ W m}^{-2}$, or $L = 5 \times 10^{14} L_{\odot}$ if $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and $q_0 = 0.1$. For $q_0 = 0$ or $q_0 = 1$ the values for L would be $7 \times 10^{14} L_{\odot}$ and $1 \times 10^{14} L_{\odot}$ respectively. More extensive IR observations would test the suitability of the spectral indices used above, and provide a better estimate of the luminosity. Our estimate will be too large if the broad bandpass $2.2 \mu\text{m}$ ($\Delta\lambda = 0.4 \mu\text{m}$) observation is strongly affected by H β and OIII, which are redshifted to just inside the bandpass. On the other hand, our luminosity estimate will be too small if the spectral distribution of Q 0420-388 continues its rise into the IR. If the spectrum rises as $\nu^{-1.1}$ out to $10 \mu\text{m}$, it will be 4 mJy (12.2 mag) at $3.5 \mu\text{m}$ and 13 mJy (8.7 mag) at $10 \mu\text{m}$, and the luminosity will be $8 \times 10^{14} L_{\odot}$. If Q 0420-388 rises into the IR as rapidly as 3C232, it will be $\sim 20 \text{ mJy}$ at $10 \mu\text{m}$ and the minimum total luminosity will be $13 \times 10^{14} L_{\odot}$. Detections at these low flux levels should be

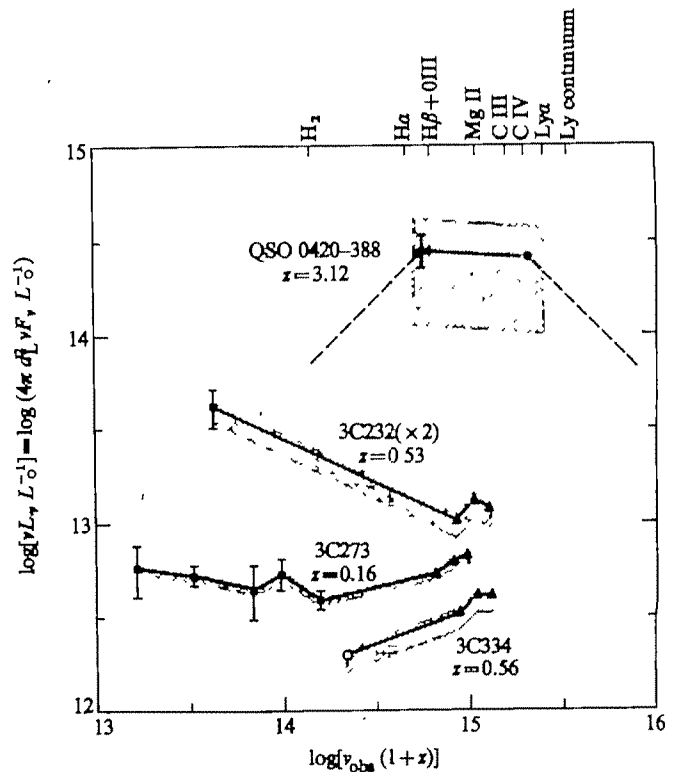


Fig. 1 Luminosity of Q 0420-388 and comparison quasi-stellar objects. The luminosity is expressed in solar units; the frequency is the rest frequency. The data points and connecting lines are drawn for $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$ and $q_0 = 0.1$. The edges of the shaded zones indicate the maximum luminosity, when $q_0 = 0$, and the luminosity when $q_0 = 0.5$. For Q 0420-388 the $\lambda_{\text{rest}} = 1,475 \text{ \AA}$ point is from Osmer and Smith¹; the datum at $\lambda_{\text{rest}} = 0.53 \mu\text{m}$ is from this paper. The dashed lines show the spectral behaviour assumed in calculating the luminosity. For the comparison quasars, Δ are UVB observations from the compilation of Smith-Haenni⁷, and $\blacksquare$ are from Rieke and Low⁵. The $\lambda_{\text{rest}} = 1.41 \mu\text{m}$ point for QSO 3C334 is a $2.2 \mu\text{m}$ observation given by Oke *et al.*⁶.

possible with long integrations on large, low-background Southern Hemisphere telescopes.

The obvious sources for comparison with Q 0420-388 do have spectra that rise into the IR. The most luminous galaxy, Markarian 231, has a bolometric luminosity of $\sim 4 \times 10^{12} L_{\odot}$, and the most famous quasar, 3C273, has a bolometric luminosity of $\leq 3 \times 10^{13} L_{\odot}$ (ref. 4). The most luminous quasar observed by Rieke and Low⁵, 3C232, can be (generously) estimated to have a bolometric luminosity of $\sim 2 \times 10^{14} L_{\odot}$, assuming that at IR wavelengths longer than $10 \mu\text{m}$ it is similar to 3C273.

The luminosity of Q 0420-388 seems to be temporally sustained. From spectrophotometric and near IR studies of 28 QSOs, Oke *et al.*⁶ argued that a steep rise into the IR ($\alpha < -1.5$) might be a necessary but not sufficient condition for rapid variability of a QSO, and that if such variation occurred, the entire UV-near IR spectrum rose or fell. The visible-IR spectral index of Q 0420-388, $\alpha = -1.1 \pm 0.2$, is shallower than the $\alpha < -1.5$ cut-on value. Moreover, the persistence of Q 0420-388 on the Whiteoak extension, on the ESO B plate series, and on the discovery plates of Osmer and Smith suggests its relative constancy. A most important finding is that the quasar is evident on seven plates in the Harvard collection (ranging back to 1936) and there is no conclusive evidence for its having varied by more than $\sim 1/2 \text{ mag}$. Thus, the luminosity may be constant, at least for time scales of several years. Additional IR and optical observations to establish the temporal behaviour of Q 0420-388 are clearly important for defining the energy requirements of the source.

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Low energy neutrino elastic scattering and supernova explosions

COLGATE AND WHITE¹ have proposed that neutrinos could transport energy outwards during the gravitational collapse of a star, thus initiating a supernova explosion. Detailed calculations by Wilson² seemed to rule out this possibility, but Freedman³ pointed out in the context of unified gauge theories of weak and electromagnetic interactions that there could be both a large neutrino-neutron elastic scattering cross section and a very large coherent neutrino-heavy nucleus cross section. Wilson⁴ has recently recalculated the collapse of an iron core formed at the end of a stellar evolution from massive stars and shown that the calculations do simulate an explosion, and that a stronger explosion results from smaller coherent scattering off heavy nuclei. This surprising result arises because a smaller cross section allows more neutrinos to escape, lowering the pressure and thereby letting the neutron core reach a higher density before the outward shock wave is formed. We have calculated neutrino-nucleon and neutrino-heavy nucleus neutral current cross sections in weak SU(2)×U(1) and compared with those of the standard unified theory. Important differences are found which could make simulated supernova explosions easier to obtain. Possible experiments to test the two theories are outlined here.

The theory of neutral weak currents which agrees with all high energy neutrino experiments is the renormalisable and unified spontaneously broken gauge theory based on the group SU(2)×U(1) (ref. 5). This provided the basis for the recent calculations by Wilson⁴ and others^{6,7}. We have suggested previously an alternative theory⁸ based on the same group which we call weak SU(2)×U(1) (as it does not involve the photon) and which is very hard to distinguish from the Weinberg model⁵ in high energy neutrino scattering. Nevertheless, there are marked differences which should have a significant effect on the supernova calculations. In particular, in weak SU(2)×U(1) there is essentially no coherent scattering of neutrinos off heavy nuclei whereas the neutrino-neutron cross section is changed relatively little.

We shall now briefly derive these results, using the notation in ref. 8. In unified SU(2)×U(1), the neutral weak hadronic current is

$$J_\mu = A_\mu^3 + (1 - 2 \sin^2 \theta_w) V_\mu^3 - 2 \sin^2 \theta_w V_\mu^S \quad (1)$$

This gives the matrix elements for neutrino-nucleon scattering

at small momentum transfer

$$\langle n | J_\mu | n \rangle = -\bar{u}_n (\gamma_\mu + g_A^3 \gamma_\mu \gamma_5) u_n \quad (2a)$$

$$\langle p | J_\mu | p \rangle = \bar{u}_p [(1 - 4 \sin^2 \theta_w) \gamma_\mu + g_A^3 \gamma_\mu \gamma_5] u_p \quad (2b)$$

where $g_A^3 = -1.25$ is the isovector axial renormalisation factor. Experimentally $\sin^2 \theta_w \approx \frac{1}{4}$ and so equation (2b) predicts that the proton neutral current is nearly pure axial. For $\sin^2 \theta_w = \frac{1}{4}$ the nonrelativistic cross sections in the Weinberg model are then

$$\sigma(\nu n \rightarrow \nu n) = G^2 p^2 (1 + 3(g_A^3)^2) / 4\pi \quad (3a)$$

$$\sigma(\nu p \rightarrow \nu p) = 3G^2 p^2 (g_A^3)^2 / 4\pi \quad (3b)$$

where p is the neutrino momentum. (In refs 6 and 7 it was assumed that $g_A^3 = -1$, thus reducing the cross section (3a) by about 30%.)

For neutrino scattering off a nucleus with Z protons and N neutrons equation (1) gives

$$\begin{aligned} \langle (Z, N) | J_\mu | (Z, N) \rangle \\ = (1 - 4 \sin^2 \theta_w) Z - N + \text{noncoherent terms} \end{aligned} \quad (4)$$

This shows that the neutrino-nucleus cross section in the Weinberg model should have a large coherent part which is proportional to N^2 for $\sin^2 \theta_w = \frac{1}{4}$. Note that this coherent contribution in this model happens to be just the quantity Q_w that should have been measured as a parity violation in atomic bismuth⁹. It was the failure to observe this effect which led to alternative versions of the weak current.

In weak SU(2)×U(1) the vector and axial current are interchanged. Thus instead of equation (1)

$$J_\mu = V_\mu^3 + (1 - 2 \sin^2 \theta_w) A_\mu^3 - 2 \sin^2 \theta_w A_\mu^S \quad (5)$$

There is now no vector isoscalar component (in Freedman's³ notation $a_0 = 0$) and so instead of equation (4)

$$\langle (Z, N) | J_\mu | (Z, N) \rangle = Z - N + \text{non-coherent terms} \quad (6)$$

Instead of equations (2a) and (2b) we have

$$\langle n | J_\mu | n \rangle = -\bar{u}_n [\gamma_\mu + g_A^3 \gamma_\mu \gamma_5 (1 - 2(1 - \lambda) \sin^2 \theta_w)] u_n \quad (7a)$$

$$\langle p | J_\mu | p \rangle = \bar{u}_p [\gamma_\mu + g_A^3 \gamma_\mu \gamma_5 (1 - 2(1 + \lambda) \sin^2 \theta_w)] u_p \quad (7b)$$

where $\lambda = g_A^S / g_A^3$ and g_A^S is the isoscalar axial renormalisation factor. If axial charge is a good quantum number $g_A^S = g_A^3$; on the other hand the quark model¹⁰ gives $g_A^S = \frac{2}{3} g_A^3$.

In terms of the ratios R_n and R_p of νn and νp cross sections in weak SU(2)×U(1) compared with those of unified SU(2)×U(1) above in the nonrelativistic limit, we have for $\sin^2 \theta_w = \frac{1}{4}$, and $\lambda = 1$

$$R_n = 1, \quad R_p = 0.21 \quad (8a)$$

and for $\sin^2 \theta_w = \frac{1}{4}$, $\lambda = \frac{2}{3}$

$$R_n = 0.7 \quad R_p = 0.25 \quad (8b)$$

(We take the quantity $\kappa = m_z \cos \theta_w / m_w$ of ref. 8 to be approximately given by $\kappa = 1$.) Therefore from equations (6)–(8), in weak SU(2)×U(1), neutrino-neutron cross sections are relatively unaffected while neutrino-proton cross sections are reduced by a factor of 4 or 5 on account of a pure axial current being replaced by a nearly pure vector current. There is very little coherent scattering and then only for heavy nuclei with $Z \neq N$. Spin $\frac{1}{2}$ nuclei with $Z = N \pm 1$ scatter neutrinos like protons or neutrons respectively.

These predictions should change the character of the supernova calculations. The most important effects of neutrinos in the calculations based on the Weinberg model come from neutrino-electron, neutrino-neutron and neutrino-heavy nucleus collisions^{6,7}. Energy is transferred most effectively in νe^- scattering and this is unchanged (neglecting the electron mass) in weak SU(2)×U(1) (ref. 8). The neutrino opacity in the neutron core is also relatively unchanged—the neutrino may

have a slightly longer mean free path in the neutron core—but what is drastically changed is the ability of the neutrino to scatter from heavy nuclei. There is now a much smaller opacity gradient between the neutron core and the $A > 1$ shell and therefore neutrinos will be able to escape through the $A > 1$ shell much faster: this could make explosions easier to obtain as higher densities in the core will be possible.

Finally, we should point out that it would be of great importance to decide experimentally between the two types of theory. Freedman³ suggested that an experiment on coherent neutrino-heavy nucleus scattering was possible by detecting the recoil nucleus, using neutrinos in the 100–200 MeV range from a 'meson factory'. Certainly a large coherent cross section would clearly discriminate in favour of the Weinberg theory or one with similar structure.

An alternative possibility is suggested by equation (8). It should be possible to measure $\nu p \rightarrow \nu p$ in the non-relativistic region at a reactor such as ILL, with a good high intensity point source of neutrinos. Recoil protons could be detected in either gas or liquid targets. If the experiment is performed it should be relatively easy to determine from the size of the cross section whether the amplitude is predominantly Gamow-Teller, as it is in the Weinberg theory for $\sin^2 \theta_w = \frac{1}{4}$, or Fermi and, therefore, 4–5 times smaller ($3(g_A^2)^2 = 4.7$) as it is in weak $SU(2) \times U(1)$.

Note added in proof. Weak $SU(2) \times U(1)$ predicts an asymmetry in polarised electron-deuteron scattering which is smaller than that observed in the recent SLAC experiment¹¹. On the other hand, the standard model is not in agreement with the Oxford and Washington experiments in bismuth vapour⁹. This confused situation makes it even more essential to measure the elastic neutrino-proton and neutrino-heavy nucleus cross sections at low energies, so that a clean determination of the weak hadronic neutral current can be made in this energy range.

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Photofixation of carbon dioxide to formic acid *in vitro* using water as hydrogen source

THE function of photosynthesis *in vivo*, including subsequent dark reactions, is defined as the reduction of carbon dioxide coupled with oxidation of water to produce organic compounds. We now report the first example of a mimicking of the function of photosynthesis based on electron transport sensitisation^{1–4}. Although the product is not carbohydrate, we consider that the present reaction system consists of (1) electron transfer from the singlet excited state of an aromatic hydrocarbon such as pyrene(Py) or perylene(Pe) to an electron acceptor such as 1,4-dicyanobenzene(DCB) or 9,10-dicyanoanthracene(DCNA) in polar media; (2) successive electron transfer from the acceptor anion radical to carbon dioxide; (3) further reactions of carbon dioxide anion radical as demonstrated in

Table 1 Photochemical formation of formic acid sensitised by Pe-DCNA system

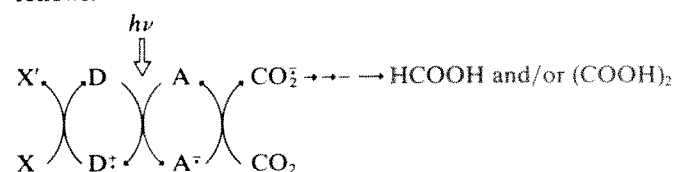
	Pe	DCNA	HCOOH	Yield (%)	
		(mg per 100 ml)		vs Pe	vs DCNA
I	6.6	15.9	3.2	270	100
II	6.2	17.0	~0	~0	~0
III	5.5	15.0	5.9	590	195
IV	1.6	4.3	1.3	450*	150*

A solution containing Pe and DCNA was irradiated (wavelength > 313 nm, runs I, II, IV, panchromatic irradiation, run III) by a 300 W high pressure Hg-arc lamp under CO_2 gas bubbling for 8 h (runs I, II, III) or 20 h (run IV). Formic acid in the reaction mixture was converted to the butyl ester and determined by gas-chromatography (polyethylene glycol 20 M 53°C). Runs I, III, IV, $\text{CH}_3\text{CN}:\text{H}_2\text{O} = 95:5$; run II, absolute CH_3CN .

* The turnover numbers for Pe and DCNA were calculated as 6.5 and 12.8, respectively, from the decomposed amounts of the sensitizers during photoirradiation determined spectroscopically.

electrolytic reduction of carbon dioxide to formic acid and oxalic acid^{5,6}.

The reaction process will then be expressed schematically as follows:



Photochemistry of carbon dioxide in condensed phase has been little studied, and the first example was the reductive coupling of aromatic hydrocarbon anion radicals with carbon dioxide to give dihydroaromatic carboxylic acids⁷. If the anion radical is sterically hindered so that the C-C bond formation with carbon dioxide is unfavourable, we expect electron transfer from the anion radical to carbon dioxide to occur. Although we cannot confirm the energetic feasibility of the electron transfer process, the available redox properties of relevant species suggest that it is a good possibility ($E_{1/2}(\text{CO}_2) = -2.15$ V (ref. 5), $E_{1/2}(\text{DCB}) = -2.00$ (ref. 8) ~ -2.48 V (ref. 9). There are no available directly comparable data for DCNA; however, the more negative value of $E(\text{A}/\text{A}^-)$ for DCNA (-0.82 V (ref. 10)) than that for DCB (-0.65 V (ref. 11)) would indicate the stronger reducing power of DCNA^- . Furthermore, the reductive fixation of CO_2 *in vivo* is coupled with the oxidation of NADPH which is apparently a weaker reducing agent than DCB^- ($E(\text{NADP}/\text{NADPH}) = -0.32$ V (ref. 12)). Thus, we carried out photoinduced electron transfer reactions in the atmosphere of carbon dioxide. The results are presented in Table 1 and 2.

The important results are summarised as follows. (1) An aqueous polar solvent is the essential requirement for the reaction. When dry acetonitrile was used, formic acid was scarcely produced (Table 1, run II, Table 2, run III). Furthermore, the yield of formic acid increased with the concentration of water (Table 2, runs, I, II). (2) As a matter of course, carbon dioxide is indispensable as the carbon source of formic acid (Table 2, run VI). (3) Qualitative confirmation of formic acid formation in other solvents such as aqueous methanol and aqueous DMF by means of spot tests¹³ indicated that these organic solvents did not play an essential part in reducing carbon dioxide. The role of organic solvents is assumed to dissolve D and A. (4) The D-A pair was indeed recycled, as evidenced by a yield of formic acid higher than 100% based on the initial concentration of D and A (Table 1, runs, I, III, IV). To recycle the electron transport system, another reducing species (X in the reaction scheme above) is required. We cannot yet draw any conclusion on the nature of X by which D is reproduced from D^+ , but probable candidates are OH^- , HCO_3^- and/or HCOOH . Iodometry of the reaction mixture (Py-DCB-TPA in $\text{CH}_3\text{CN}:\text{H}_2\text{O} = 5:1$, panchromatic irradiation for 8 h under a CO_2 pressure of

Table 2 Photochemical formation of formic acid sensitised by Py-DCB-TPA system

	[Py]	[DCB] ($\times 10^{-2}$ mol l $^{-1}$)	[TPA]	[HCOOH] ($\times 10^{-4}$ mol l $^{-1}$)
I	1.9	5.4	5.2	5.2
II	1.7	6.0	4.5	17
III	1.8	5.4	5.7	~0
IV	1.9	—	6.0	9.6
V	1.8	5.8	—	8.6
VI	1.9	5.6	5.4	0

A pressure tight pyrex vessel containing a solution of sensitisers was pressurised (4 kg cm $^{-2}$) with CO $_2$ (runs I-V) (or N $_2$) (run VI) and irradiated by a 300 W high pressure Hg-arc lamp for 8 h. Formic acid in the reaction mixture was esterified to the benzyl ester and determined gas-chromatographically. Runs I, VI, CH $_3$ CN:H $_2$ O = 95:5; runs II, IV, V, CH $_3$ CN:H $_2$ O = 5:1; run III, absolute CH $_3$ CN.

5 kg cm $^{-2}$) confirmed the formation of peroxide, suggesting that D $^+$ was reduced to D at least in part by the reaction, OH $^- \xrightarrow{e^-} 1/2$ H $_2$ O $_2$. (5) The turnover numbers of sensitiser (2X[HCOOH]/ Δ [sensitiser]) were determined to be 6.5 for DCNA and 12.8 for Pe (Table 1, run IV).

We have not yet determined the quantum yield of formic acid production, as the kinetics of the total reaction is complicated by several possible secondary reactions. A very coarse estimation of apparent efficiency could, however, be made by dividing the product by the energy input. The result shows that $\sim 3 \times 10^2$ mol of photon is required to reduce 1 mol of CO $_2$. This efficiency is extremely poor compared with the natural realisation of light conversion by photosynthesis. The possible reasons are: (1) antenna pigments are absent so that the cross-section of photoabsorption is small. (2) As the oxidised and the reduced products exist together in a homogeneous solution, back-reactions such as D $^+ + A^- \rightarrow D + A$, CO $_2^- + D^+ \rightarrow$ CO $_2 + D$ and others will come into effect. (3) The product is not stable and reactions such as HCOOH $\rightarrow$ CO + H $_2$ O or CO $_2 + H_2$, HCOOH + D $^+ \rightarrow$ H $^+ + 1/2$ H $_2 +$ CO $_2$ would reduce the apparent yield¹⁴. Separation of oxidising and reducing compartments by electron transport membrane would be a promising strategy to refine the present mimicked photosynthesis.

In conclusion, the results in Tables 1 and 2 indicate that the main overall reaction is the photoreduction of carbon dioxide with water as follows:



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Io-U dating of the Ouljian stage from Torre Garcia (southern Spain)

IN this note we discuss the reliability of the Io-U dating method and report on the age of fossils from the Ouljian stage, outcropping along the Mediterranean coast in southern Spain. That this method can produce good results is evident from the fact that the measured ages for these fossils, all from the same stratigraphic horizon, show a standard deviation of only 6,100 yr, which is well within our precision limits. The mean age of the samples is 98,000 yr.

The dating of mollusc shells by the Io-U method has been controversial; some of the ages obtained in this way have been incompatible with other age data, and some samples from the same epoche have shown a considerable scatter in their ages. It seemed that the 'closed system' condition, a requirement for successful application of this method, is frequently violated. However, certain indications, such as the absence of ^{232}Th , and $^{234}\text{U}/^{238}\text{U}$ ratios compatible with the calculated Io-U ages suggest that little or no contamination occurred. Szabo and Rosholt¹ have proposed a model to correct for the suspected open character of the system, this model has been criticised by Kaufman *et al.*² and it seems to be of only limited interest for this study.

Although the value of the Io-U dating method is in doubt, it is the only technique available for dating events occurring between 30,000 and 250,000 yr ago. In order to test the reliability of the Io-U method we have analysed six mollusc shells which are contemporaneous—that is, they come from the same stratigraphic horizon, the Ouljian stage. The best way to check the reliability of the method is to see if the measured ages show a significant dispersion and if the calculated absolute ages are reasonable. The rocks of the Ouljian stage outcrop in Spain and North Africa; it is important to establish an absolute age for this stage to study the secular changes in the level of the sea during the Quaternary. Because the outcrops of the Ouljian at both sides of the sea of Alboran occur at different elevations, dating of this stratigraphic level provides information about recent tectonic movements in this region.

We report here on six shells from the Ouljian, outcropping in the region of Torre Garcia along the beach of the Gulf of Almeria. The Pleistocene geology of this region has been discussed by Overejo *et al.*³. Sandstones of the Ouljian, containing many *Strombus bubonius* and *Pectunculus* outcrop at a few points at 5 m above sea level; most of this formation is covered by recent dune sand. A unique level characterised by *Strombus* has been recognised⁴⁻⁶. This level has been correlated with the Ouljian stage in Morocco⁴ which was dated between 80,000 and 120,000 yr (refs 4, 7). One age of 80,000 yr has been published⁷ for a *Pectunculus* shell coming from the *Strombus* episode about 50 km NE from our sampling location at Torre Garcia. The six samples we analysed from the same horizon were cleaned mechanically; we removed about 1 mm of the superficial part. We worked mainly on the most massive parts of the shells of the *Strombus*, but for two shells, we have also analysed the thin section. All the samples were in good condition except samples I and III which had been attacked by marine organisms in some parts.

α spectrometry was used to measure uranium and thorium isotope abundances. Using the method of Goldberg *et al.*⁸, these elements in the dissolved samples were chemically separated by coprecipitation, followed by purification with organic solvents and electrodepositions. ^{232}U and ^{234}Th (a β emitter), were used as tracers. Results are shown in Table 1.

The ^{232}Th concentration was <0.001 p.p.m. in all samples; the ^{232}Th activity for the total sample (1-4 g) is barely resolvable above the background noise. It is also clear that, after the observed ^{228}Th signal has been corrected for background noise

Table 1 Analysis of six mollusc shells from the Ouljian stage

Sample	²³⁴ U/ ²³⁸ U	U (p.p.m.)	²³⁴ U (d.p.m. g ⁻¹)	I ₀ (²³⁰ Th) (d.p.m. g ⁻¹)	Age (yr)	% Aragonite
I*	1.08 ± 0.04	0.607	0.491	0.286	93,000	>90
I†	1.12 ± 0.06	0.789	0.659	0.420	108,000	>90
II	1.075 ± 0.04	0.514	0.412	0.253	102,000	Argonite > calcite
III	1.09 ± 0.04	0.846	0.689	0.414	98,000	Argonite < calcite
IV	1.09 ± 0.04	0.843	0.686	0.403	95,000	100
V*	1.12 ± 0.03	0.662	0.553	0.306	88,000	>90
V†	1.13 ± 0.04	1.03	0.875	0.534	101,000	>90
VI	1.08 ± 0.05	0.469	0.378	0.229	99,000	100

From the age values shown the mean is 98,000 yr ± 6,100 yr.
* From massive part of the shell.
† From thin part of the shell.

and the ²²⁸Th introduced via the tracer ²³²U, there is no ²²⁸Th in our samples. Of course, this is to be expected, because ²²⁸Th is a radiogenic daughter of ²³²Th. However, ²²⁸Ra (period 6.74 yr), occurs in this decay chain, and in certain situations this element is very mobile and thus the absence of ²²⁸Th suggests that no recent contamination has taken place at least by radium. Uranium concentrations range from 0.6 to 1.2 p.p.m. In shells I and V we have analysed the thin parts where the U concentration is significantly greater than in the remainder of the shell. The ratios ²³⁴U/²³⁸U are always less than 1.15 which corresponds to the value in present-day seawater. The measured ²³⁴U/²³⁸U ratios are compatible with the calculated ages for the samples to within our precision limits. The ratios in the thin parts of the shells I and V are somewhat larger than for the massive samples. The greater part of each sample is aragonitic, with calcite content less than 10%; two samples (II, IV) contain more than 10%.

The uranium concentration in the shells of living molluscs is very low, often less than 0.1 p.p.m. but in the fossil shells it can be several p.p.m. The concentrations we have measured are not very high but uranium enrichment has obviously taken place. This occurs very rapidly after the animal has died⁹: Kaufman *et al.*² have confirmed this conclusion by a statistical analysis of the uranium concentration in mollusc shells of known ages. The uranium enrichment in the shells of molluscs which have just died seems to be related to oxidation-reduction reactions occurring during the decomposition of organic material of the mollusc body. Blanchard *et al.*¹⁰ have also shown that the superficial parts of the shell are selectively enriched—conforming to our observations.

This post-mortem enrichment in uranium may cause errors of a few thousand years in dating and is thus of no great importance for our purpose. The samples may be affected by other contamination events at later stages, however. But in the present case we have no clear indication of this. For instance, contact with freshwater could cause changes in the ²³⁴U/²³⁸U ratio and in the ²³²Th abundance. Our samples (V, I) show that the uranium enrichment of the thin parts of the shells is associated with an increase of the I₀ activity. No ²³²Th activity was detected, however, so the increased I₀ activity is probably due to *in situ* production. The calculated ages of the thin parts of the shells tend to be somewhat greater than the age of the massive parts; because I₀ is not very mobile these age differences could be caused by an uranium loss occurring mainly in the superficial part. The ²³⁴U/²³⁸U ratios of all the samples are (within error limits) compatible with the I₀-U ages and hence continental water uranium contamination with its much larger ²³⁴U/²³⁸U ratio is not likely. It is also obvious that the calcite content of the shells does not show any correlation with the ages. Calcitic samples, which often contain less uranium (this is not the case here) give ages very similar to those for aragonitic samples².

Ignoring the 108,000 yr age for the thin part of shell I, which could be the result of a small loss of uranium from the outer part,

we note that the ages obtained from the remaining seven samples range from 88,000 to 102 000 yr. The mean age of these seven samples is 97,000 yr with a standard deviation of 4,900 yr. Our experimental precision is about 7%, so our results are well within precision limits. Including the 108,000 yr age, the mean age would only increase to 98,000 yr with a standard deviation of 6,100 yr. These results are in general agreement with previously obtained ages for the Ouljian⁷ but show far less scatter.

These results indicate implicitly that 98,000 yr ago the sea level was about 5 m higher, relative to the present location of the Ouljian outcrop from which our samples came. This is in contrast to sea level investigations which have indicated that the worldwide palaeo-sea level was 10 to 20 m below the present level at about 100,000 yr ago. The accuracy of our work rules out the possibility that the true age of our samples is 125,000 ± 10,000 yr, an age at which sea level was higher than today. We interpret our results to mean that the coast of the Gulf of Almeria has experienced a net upwards vertical movement of 5 m with respect to sea level and a net total uplift of 15–25 m relative to a fixed reference frame, since 98,000 yr ago, that is, 15–25 cm per thousand years.

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Dissipation of tidal energy by Antarctic ice shelves

FLEXING of ice shelves may dissipate enough tidal energy to have a significant effect on the Moon's retreat from the Earth. It is generally agreed that tidal friction slows the rotation of the Earth and decelerates the Moon in its orbit¹. The total energy dissipated can be calculated from observations of the Moon's motion and agrees closely with estimates of the gravitational work done by the Sun and Moon on the ocean tidal bulge². Therefore dissipation of tidal energy is considered to occur mainly in the ocean with small upper limits being set on energy losses in the Earth's mantle and core. Because the amount of tidal dissipation in shallow seas³ and internal tides⁴ is uncertain it is of interest to search for additional dissipative mechanisms. In this note the possibility of tidal bending of ice shelves is examined.

An ice shelf is an ice sheet floating on the sea. In Antarctica ice shelves cover an area of $>1.5 \times 10^6$ km². Thicknesses range from 100 m at the seaward ice front to $>1,800$ m at the grounding line where the ice is attached to land⁵. Ice shelves rise and fall freely with the tide except in a bending zone near the grounding line where the ice shelf deforms. A discussion of different types of grounding line and their expected dynamic behaviour has been given by Hughes⁶. An estimate of the rate at which energy is dissipated in the bending zone can be made by assuming that ice is deforming plastically. A transient creep process with parabolic strain hardening is thought to be appropriate for stress rates occurring in tidal flexure^{6,7}. Using equations derived by Holdsworth⁷ for the surface bending stress σ , average strain rate $\dot{\epsilon}$, and exponential damping factor λ ($=\lambda p/2$ in Holdsworth's notation), the following expression can be derived for the rate at which energy is dissipated;

$$\dot{E} = \frac{2hL\sigma\dot{\epsilon}}{\lambda} = 1.24 \times 10^7 h^{29/30} \xi^{1/2} \dot{\epsilon}^{16/15} L \text{ watts}$$

where $2h$ is the ice thickness at the grounding line, L the total length of hinge line and ξ the tidal amplitude. This simply assumes that energy is dissipated at the rate of $\sigma\dot{\epsilon}$ per unit volume and that the total volume of ice affected is $2hL/\lambda$. Taking values for the Antarctic of $2h$ as 500 m, L as 16×10^3 km (ref. 8) and a semi-diurnal tidal amplitude ξ as 1 m gives $\dot{E}$ as 2.0×10^{12} W.

There are many uncertainties associated with this figure. A value for the maximum surface bending stress has been used as though it applied throughout the whole flexing region; however, on the assumption that the bending strain decays linearly towards the neutral axis the stress will decay parabolically⁷ reducing the energy dissipated by a third. The various types of grounding line are expected to have different stress patterns, but as the calculation here is for the case of least average tidal flexure strain⁶ the total dissipation is probably an underestimate. Crevasseing on the top and bottom may reduce the dissipation considerably. The length of the grounding line L includes both ice shelves and outlet glaciers and was derived⁸ from maps published up to 1965. Perhaps the greatest change to the mapped coastline of Antarctica since then has occurred on the Ronne Ice Shelf⁵. However, although the position of the coast has been altered, there has been little change in the actual length of coast. The greatest error probably lies in not considering ice rise boundaries, and thus the figure used should be regarded as a lower bound. There is more uncertainty in the value used for the mean thickness of ice at the hinge zone. Although the major ice shelves have at least some depth soundings, data for grounding line regions are sparse. Using published ice thickness contour maps for the Ross Ice Shelf⁹ and the Ronne Ice Shelf⁵ (excluding the Filchner Ice Shelf), their mean thickness at the grounding line can be calculated as 610 m and 990 m respectively. These

two ice shelves contain a quarter of the total length of grounding line. Another approach is to take the mean thickness of all Antarctic ice shelves, 390 m (ref. 8), so that by assuming a value for the mean thickness at the ice front where the ice is thinnest the mean grounding line thickness can be estimated. Taking a value at the ice front of 200 m gives the mean thickness at the grounding line to be 580 m. So again, the actual figure of 500 m used in the calculation probably errs on the side of caution. The dissipation rate of 2.0×10^{12} W is unlikely to be underestimated because of errors in h and L . A reasonable error, considering the competing nature of all the uncertainties, might be of the order of 50%.

One way of checking whether this amount of dissipation by bending is feasible would be to estimate the total tidal energy flux under ice shelves. In general, the energy flux per unit area perpendicular to the direction of propagation is given by $\frac{1}{2}\rho g u \xi \cos \theta$ where ξ and u are the amplitudes of tidal height and current respectively. For a progressive wave $\theta = 0$ and the maximum current flow inwards occurs at high tide; for a standing wave $\theta = \pi/2$ and there is no net energy flux. Again, because of the almost complete lack of relevant data for Antarctica it is impossible to perform a meaningful calculation. However, if the energy flux under a section of the Filchner Ice Shelf, where a value of tidal current of 0.1 m s^{-1} is assumed¹⁰ for a site where the depth of the water column under the ice front is 600 m (ref. 11) is taken as typical for the rest of Antarctica, then for $\theta = 0$ the required energy flux would be provided by only 6.6×10^3 km of ice front. It is therefore reasonable to say that there is probably enough incoming energy to allow the dissipation rate calculated for tidal bending.

To account for the astronomically observed lunar acceleration a dissipation rate of $5.7 \pm 0.5 \times 10^{12}$ W is required². This is close to the estimate of $5.0 \pm 0.3 \times 10^{12}$ W for the work done on the ocean surface by the luni-solar torque². The problem is to determine how the dissipation occurs in the ocean and to partition the energy loss among the mechanisms. An estimate of the frictional losses in shallow seas and continental shelves³ is $1.5 \pm 0.8 \times 10^{12}$ W. However, many of the areas where dissipation occurs are situated in high latitudes where the main tidal torque is reduced by a factor $\cos^2 \psi$ where ψ is the latitude¹². The same problem applies to ice shelf dissipation. It is uncertain how dissipation in high latitudes and in localised regions can affect the rest of the ocean tide. A more even dissipation of tidal energy would be by internal (baroclinic) tides, but an estimate of the energy loss is of the order of 10% of the total tidal dissipation⁴. After allowing for errors the total dissipation from these three mechanisms can therefore barely account for the required amount.

If tidal energy is being lost under ice shelves then one might expect to find that the age of the semi-diurnal tide is high. The age of the tide is the time interval between the time of new or full Moon and the time of the spring tide. Other areas of the ocean where the age of the tide is greater than 60 h are associated with strong tidal dissipation¹³. There are few reliable tidal records from Antarctica but the majority show ages greater than 60 h. The most detailed analysis has been carried out by Cartwright¹⁴ on records gathered along the west coast of the Antarctic Peninsula for observation periods of up to 9 yr. The results clearly show increasing age of tide with latitude, culminating in ages of 165 h for tidal records obtained on George VI Ice Shelf. Gravimetric readings on the Ross Ice Shelf also show a very high age of the semi-diurnal tide but with small amplitudes¹⁵. Conversely, the lowest age found in Antarctica (17 h) has been measured on the edge of the Filchner Ice Shelf¹⁶. This may be an indication of the complexity of the interaction between ice shelves and tides especially near critical latitudes where tidal and inertial frequencies are equal¹⁴. In principle the energy flux under an ice shelf could be calculated from simultaneous measurements of tidal amplitude and current. It would then be possible to decide the importance of tidal dissipation both by bending and by frictional losses along the bottom of the ice shelf and the bottom of the sea.

If ice shelves form a major sink for tidal energy then there are implications on a geological time scale. It might be expected that the times of maximum slowing of the Earth's rotation would coincide with times of maximum extent of ice shelves. Within the constraints imposed by the lack of data, this seems to be the case; extensive glaciation occurs in the periods preceding 'turning points' when the Earth increases its spin velocity¹⁷.

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Melting of Antarctic icebergs

PROPOSALS for supplying water to arid regions through the towing and utilisation of Antarctic icebergs^{1–5} have generated considerable interest. Simulation studies on the towing of unprotected icebergs to southern continents⁶ suggest that the towing distance, ocean currents and the iceberg deterioration rate are of major importance. The distance and current components may be optimised by a careful choice of iceberg position, although much further study of currents in southern latitudes is required. The rate of deterioration is probably more difficult to calculate as a number of melting and calving mechanisms may be operating. In this note some initial studies of iceberg melting are reported and some of the problems involved in further investigations are considered.

Above-sea surface melting is probably due to solar radiation and wind-enhanced convection. Under-sea melting may occur through turbulent natural convection. This should be increased by iceberg surface discontinuities, iceberg wallowing or by release of entrapped air bubbles on melting. Calving could result from side undercutting by wave action⁷, differential melting along cracks and low density areas⁸, stress on underwater shelves formed through layered melting⁹ or thermal stress-induced disintegration following calving in warm water⁶.

If under-sea melting is an important ablation mechanism, then one or more of the following three effects might be detectable. (1) The lighter freshwater could rise up the sides of the iceberg with little mixing to produce a pool of less saline water. If this happens then it would be possible for meltwater to be collected in warm waters by building a shallow pen around the iceberg. (2) The lighter freshwater may entrain warmer, saltier water from the surrounding sea and thus be responsible for considerable vertical transport of deep water and nutrients¹⁰. (3) Melting could occur in a stratified manner and the freshwater may then disseminate in layers. This type of melting has been detected in model studies⁹.

Thus evidence of gross salinity variation near an iceberg would support mechanism (1), and a temperature rise would support mechanism (2). To test for these two mechanisms,

salinities were measured around three icebergs in the northern Ross Sea/Southern Ocean. (Mechanism (3), suggested only recently⁹, was not tested. The salinity changes involved would probably not be detectable with the small portable salinometer used.)

All measurements were made from the 18-m yacht Solo using a Yellowsprings Instruments portable salinity/temperature meter (sensitivity, $S = \pm 0.05\%$), equipped with 200 m of cable. Salinities were recorded in the open sea in the following conditions. (1) Motoring as close as safety permitted (20 m from underwater ram) on the lee side of an iceberg; as the yacht drifted slowly downwind, measurements were taken continuously. (2) Circling an iceberg (speed ~ 2 knots) at known distances (closest weather side measurements were at 60 m). The meter was standardised at ambient temperature before use with a standard seawater solution¹¹. Salinity data were gathered at distances of 20 m to 800 m (range under values) and at depths of 5, 10, 25, 60, 100 and 200 m. A correction for drag on the weighted probe was not applied but depths are probably overestimated by no more than 20%.

The results are shown in Table 1. No variation of salinity with distance from icebergs was found. A temperature inversion at a depth of 50–60 m around iceberg no. 3 was recorded but this extended for at least 2 km. Again, no salinity variation with distance was detectable along this discontinuity.

It is perhaps not surprising that there was no evidence of a salinity gradient with distance around icebergs, even in water to $+5.6^\circ\text{C}$. It seems likely that naturally positioned icebergs are not melting rapidly or that if melting is substantial then either mixing or rapid layered spreading may occur. If only little melting is occurring this might be because of the formation of a narrow insulatory envelope of cold water. Disturbance of such an interface by iceberg towing would then increase the melting rate dramatically. Unambiguous results will probably only be achieved by using a more accurate meter very close to an iceberg (possibly a dangerous undertaking).

No salinity step structure like that observed in the Weddell Sea¹² was observed, but a more accurate meter might detect such salinity steps in this region of ocean. However, a gross thermal inversion at 60 m was observed near iceberg 3. This thermal inversion is large for waters south of the Antarctic Convergence. Similar salinity profiles without step structure have been maintained in tanks with sloping boundaries¹³. Rapid changes in surface sea temperature at the Antarctic Convergence were measured further north at 59°S , 163°E . The lack of a shallow warm surface layer in the regions studied may be due to consistently overcast, cold, foggy weather.

Table 1 Summary of observations

	Iceberg 1	Iceberg 2	Iceberg 3
Date	16/1/78	17/1/78	29/1/78
Size*	0.5 km $\times$ 0.2 km $\times$ 30 m	0.4 km $\times$ 0.3 km $\times$ 30 m	1.2 km $\times$ 0.8 km $\times$ 35 m
Latitude $^\circ\text{S}^\dagger$	66.8	66.8	65.3
Longitude $^\circ\text{E}^\dagger$	173.4	173.5	164.7
Sea temp. ($^\circ\text{C}$)	$-1.4^\ddagger$	$-1.4^\ddagger$	$+2.4^\S$
Air temp. ($^\circ\text{C}$)			$+5.6^\parallel$
Sea temp. ($^\circ\text{C}$)			$+1.0$
Swell height (m)	1.5	1.5	3
Swell direction	270°	270°	320°
Swell period (s)	7	7	6
Wind speed (m s^{-1})	2.5	4.5	7.5
Wind direction	270°	270°	320°
Cloud	7/8	5/8	8/8
Salinity $\%^\P$	34.5 ‡	34.5 ‡	34.8 § 35.1 $^\parallel$

* Above sea.

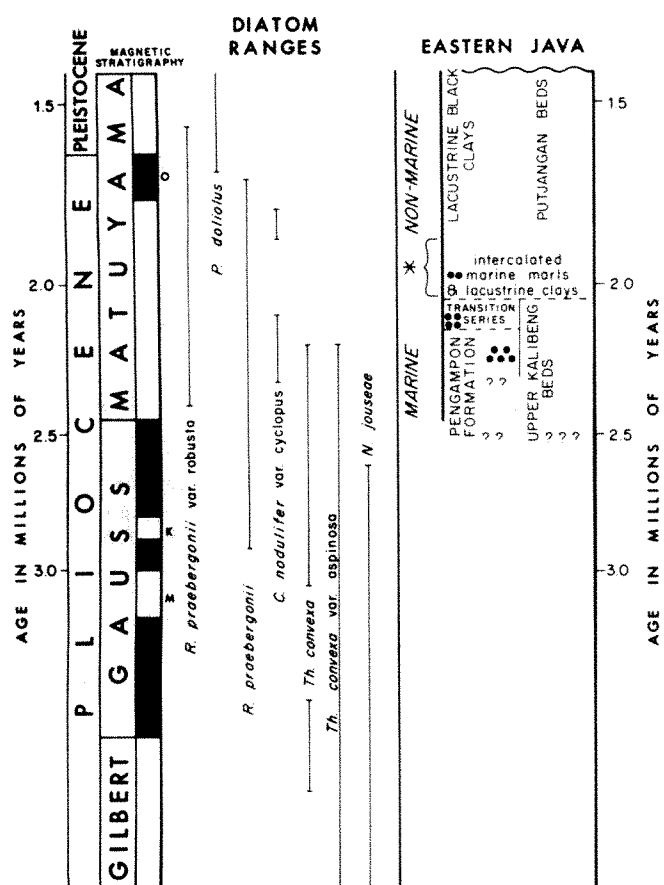
† Celestial navigation.

‡ All depths and distances.

§ 5–25 m depths, all distances.

$^\parallel$ 60–200 m depth, all distances.

¶ Measurements made to $S = \pm 0.05\%$.



however, have been attributed to the Putjangan Beds with reasonable certainty¹⁹⁻²¹.

Weidenreich²² studied the Javanese hominid fossils and suggested the following evolutionary trend, based on their morphological characteristics: *Meganthropus palaeojavanicus* → *H. modjokertensis* (which he named *robustus*) → *H. erectus*. Some hominid fossils from Late Pleistocene sediments, associated with the Ngandong mammalian fauna, correspond to an advanced *H. erectus*, or the *Neanderthal*²³. Tobias and Von Koenigswald²⁴ compared hominid fossils attributed to the Putjangan Beds with fossils from Olduvai Gorge. They concluded that *H. modjokertensis* (IV and B) is similar to *H. habilis* (Hominid 13) from the lower part of the Olduvai Bed II, while *M. palaeojavanicus* is similar, but a somewhat more primitive form than *H. habilis* (Hominid 4) from Olduvai Bed I (Fig. 1). This correlation is in agreement with Weidenreich's²² evolutionary trend of the Javanese hominids. *H. dubius*^{20,21} may represent, according to Von Koenigswald²¹, the earliest form of hominids recovered from Java. A K-Ar age of 1.9 ± 0.4 Myr has been obtained for the Putjangan Beds near Modjokerto, a few metres below the level where the skull of *H. modjokertensis* was recovered^{25,26}. Thus, the available data compiled in Fig. 1 suggest that the boundary between the Kabuh and Putjangan Beds may be tentatively placed about the middle of Bed II of Olduvai Gorge, and that the base of the Putjangan Beds in Eastern Java is older than the 1.8 Myr fossiliferous level of the Olduvai Gorge Bed I.

continental beds. As Reinhold²⁷ pointed out, the diatoms in these various units largely reflect the gradual emergence of Java. Previous work by us^{28,29} has tied Late Pliocene and Pleistocene stratigraphic events (datum levels) in the Tropical Indo-Pacific to the palaeomagnetic reversal and oxygen isotope records^{30,31}. The use of these two standards tied to diatom datum levels permits us to draw correlation lines for the Late Pliocene and Pleistocene of the Tropical Indo-Pacific with a high degree of reliability. Within this context, we studied the marine diatoms in Reinhold's original samples from the Upper Kalibeng Beds and from the marine intercalations in the overlying Putjangan Beds (Fig. 2). These were compared with diatom ranges in a central Pacific core (V28-179) which has an expanded Late Pliocene section and which has been analysed for palaeomagnetism and oxygen isotopes³¹. The lower part of the Upper Kalibeng Beds contains *Rhizosolenia praebergonii*, *R. praebergonii* var. *robusta*, *Thalassiosira convexa* and *Th. convexa* var. *aspinosa* suggesting a correlation with the basal part of the Matuyama Reversed Epoch. The upper parts of the Upper Kalibeng Beds, on the other hand, contain only two stratigraphic markers (*R. praebergonii* and *R. praebergonii* var. *robusta*), thus placing it after the last appearance of *Th. convexa* and *Th. convexa* var. *aspinosa*. The absence of *Pseudoeunotia doliolus* and of the recurring species *Coscinodiscus nodulifer* var. *cyclopus* suggests that the contact between the Upper Kalibeng Beds and the Putjangan Beds (and, thus, the transition between marine and non-marine sediments) should be dated after the last occurrences of *Th. convexa* and *Th. convexa* var. *aspinosa* and *C. nodulifer* var. *cyclopus* and before the first appearance of *P. doliolus* and reappearance of *C. nodulifer* var. *cyclopus*. Within this context, our data suggest that the base of the Putjangan Beds in Eastern Java falls between approximately 1.9 and 2.1 Myr.

As the earliest hominid fossils occur in lake sediments of the Putjangan Beds which overlay Pliocene marine sediments, early man must have migrated to Eastern Java soon after the local emergence. The age of 1.9–2.1 Myr given by diatom analysis for this emergence must be considered tentative, however, pending more detailed sampling for micropalaeontologic and palaeomagnetic study. Further, since the uplift of Java proceeded from west to east⁸ a micropalaeontological and palaeomagnetic study of the marine/non-marine transition in several sections could provide us with a more detailed history of the emergence of this island during the Late Cainozoic³¹.

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Minor metal content of surface seawater particulates and organic-rich shelf sediments

CERTAIN sediments deposited under areas of high primary productivity contain considerably more organic carbon than those laid down in other marine environments; for example, diatomaceous clays on the continental shelf off South-West Africa (Namibia) have an average of 9% organic carbon¹. Calvert and Price² have reported that these sediments also have enhanced concentrations of some trace metals, such as Cu, Zn and Pb. During the past few years we have been carrying out collections of surface (0–5 m) seawater particulates from various areas of the World oceans^{3–6}. Here, we present average data on the concentrations of Al, Cu, Zn, Pb and organic carbon in a series of particulates collected off the coast of West Africa, and compare them to organic-rich marine sediments.

Calvert and Price compared the modern environment of organic-rich sedimentation on the continental shelf of Namibia to that prevalent during the deposition of some ancient bituminous shales. According to Brongersma-Saunders^{7,8} the enrichment of Cu, Pb and Zn in many of these deposits, such as the Permian Kupferschiefer of northern Germany, is the result of their supply by plankton in regions of upwelling. However, Calvert and Price² found that the concentrations of Cu, Zn and Pb in the sediments on the South-West African shelf were not enriched to the same extent as in the Kupferschiefer, and concluded that the same mechanism of trace element deposition had not been operative in the two areas. Nonetheless, Calvert and Price² found a high degree of correlation between the Cu, Pb and Zn and the organic carbon concentrations in their sediments, and suggested that the metals were intimately associated with the organic fractions of the deposits. They stressed, however, that before the mechanism of deposition could be identified, more data were required on the relative contributions made to the total metal concentrations by biogenous, terrigenous and authigenic sources. In this respect, the concentrations of Cu, Zn and Pb in the suspended material of the overlying waters is of critical importance. Data have been

Table 1 The partial element average compositions (p.p.m.) of South-West African shelf sediments and northeastern Atlantic surface water particulates

	12 Northeastern Atlantic particulates	Diatomaceous clay South-West African shelf*
Al	9,700	15,446
Cu	112	68
Zn	225	68
Pb	50	12
Organic C	219,000	93,500

*Data from Calvert¹.

provided on the trace metal contents of plankton from various regions of the World oceans⁹, and although the metal concentrations vary considerably from one region to another certain overall enrichment trends are apparent. However, there is a general lack of information on the composition of total particulate material, a mixture of lithogenous, hydrogenous and biogenous components, from regions of high primary production.

We made particulate collections by a continuous centrifuge technique described elsewhere⁶; Cu, Zn, Pb and Al were determined by either atomic absorption or spectrographic methods, and organic carbon was measured using a C–H–N analyser. The samples selected for the sediment comparison were all collected on transects within ~500 km of the coast of West Africa between 8 and 30° N, during April, July and October 1972 and February 1973.

None of the particulates were collected off the coast of South-West Africa. However, the sample transects were chosen such that they are all within the general region of high primary production lying off the coast of West Africa (5–30° N)¹⁰. The particulates should, therefore, offer a reasonable comparison with the organic-rich South-West African shelf sediments if theories of planktonic sedimentation are to have a general ocean-wide application to the genesis of organic-rich near-shore sediments and their lithified equivalents. In other words, the two data sets, that is, those of the particulates and the shelf sediments, can be used to test the geographical independence of the general mechanisms which result in the correlation between organic matter and certain trace metals in sediments underlying areas of upwelling.

Table 2 The maximum minor element/organic carbon ratios in South-West African shelf sediments and the average ratios in northeastern Atlantic surface water particulates

Element/organic carbon (% × 10 ³)	Northeastern Atlantic particulates	Diatomaceous clays, South-West African shelf*
Cu	0.51	0.56
Zn	1.0	1.8
Pb	0.23	0.29

*Data from Calvert and Price².

The average concentrations of Cu, Zn, Pb, Al and organic carbon in the South-West Africa shelf sediments and the northeastern Atlantic particulates are given in Table 1. This shows that the sediments are not composed simply of settled undifferentiated bulk samples of the surface water particulates. For example, the sediments have a much larger 'terrestrial' component, represented by Al, and a much smaller 'planktonic' component, represented by organic carbon, than do the particulates. This is not unexpected because of the various diagenetic processes which affect the deposited sediments. However, in addition to bulk samples, Calvert and Price² have given data on the maximum concentrations of Zn, Cu and Pb in the sediments, together with that of organic carbon. If these maximum values are assumed to offer the best available estimate of the extreme situation in which the plankton have retained their original composition, they can be directly compared to the surface water particulates. Such a comparison is made in Table 2, from which it can be seen that the metal/organic carbon ratios in the particulates are similar to those in the maximum metal-enriched sediments.

The initial composition of plankton-rich surface water particulates is usually considerably modified, after deposition, by diagenetic processes. However, the data presented here strongly suggest that modern organic-rich sediments underlying regions of high primary production can receive a substantial proportion of their Cu, Zn and Pb from the sinking of relatively undifferentiated surface water particulates of which organic components,

including plankton and faecal pellets, are an important constituent.

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Diversity in constant and fluctuating environments

It has been suggested that environmental fluctuations will limit the degree to which species may be packed; the closeness of the packing increasing with increasing environmental stability¹. This theory has been challenged by the suggestion that although there is a limit to the degree of niche overlap in the real world, the limit is only sensitive to severe environmental fluctuations². The fluctuations are regarded as severe when their variance to mean ratio is greater than or equal to 0.3 (ref. 2). We report here our studies on populations of ciliate protozoa of a freshwater community maintained in temperature-controlled microcosms. Comparisons were made between the changes in diversity of the ciliates in a microcosm maintained in a fluctuating temperature and the changes taking place in a control maintained at a constant temperature. The results suggest that the diversity of the ciliates is significantly greater in the fluctuating-temperature system than in the constant-temperature system.

Samples of water were taken from Sand Loch, Aberdeenshire (grid ref. NJ 034284), and brought back to the laboratory. The water was filtered to remove large pieces of debris before being put into the experimental system. (The mesh of the filter was square, with sides of 250 μm .) Two sets of samples were collected, the first in January 1978 (experiment 1) and the second in April 1978 (experiment 2).

The water samples were maintained in batch culture in four 20-l flasks, which were kept in one of two water baths, the temperature of each being independently thermostatically controlled. The fluctuating environment was created in one bath by daily variations in the temperature (Figs 1 and 2) ranging over 2–12 °C, with a complete cycle of variation occurring every 2 d. The control system was maintained at 7 °C. In each system, samples were taken from one flask while the other acted as a reservoir to maintain a constant culture volume in the sampled vessel. A sample of 0.25 l was taken from each system daily and sedimented overnight according to the method described by Lund, Kipling and LeCren³. The ciliates present in each sample were identified (to genus level) and counted during the day after

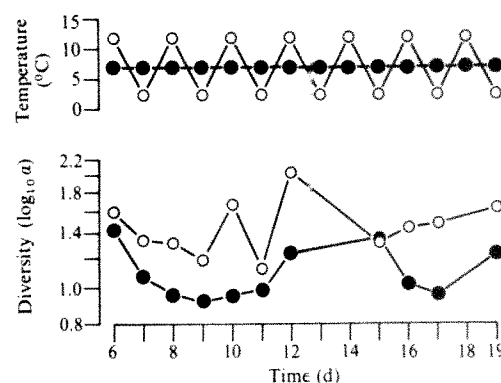


Fig. 1 Changes in temperature and diversity with time during expt 1. The lower graph shows the change in diversity ($\log_{10} \alpha$) with time (d) for the fluctuating-temperature system (○) and for the constant-temperature system (●). The upper graph shows the change in temperature (°C) with time (d) for the two temperature regimes, fluctuating temperature (○) and constant temperature (●).

sampling. The diversity of the ciliates present in the cultures was estimated using the log-series parameter, α (Figs 1 and 2). This index was used in preference to the Shannon–Wiener information index⁴ because the former does not overemphasise the presence of the commonest taxa to the same extent as the latter^{5,6}. Also, it has been shown that the site-discriminating ability of α is superior to the information index⁵ and is, therefore, more applicable in this type of investigation.

Determination of the between-system to within-system variance (Table 1) showed that variation between the diversity of the ciliates in the two temperature regimes was significantly greater than that within each of the two systems in both experiments ($P < 0.01$ in experiment 1, $P < 0.05$ in experiment 2). The diversity of the two ciliate populations did not differ significantly initially, but it was found that over a period, the diversity of the ciliate populations in the fluctuating-temperature system was significantly greater than that of those maintained in a constant temperature (in both experiments $P < 0.01$). A Spearman rank correlation showed that in the first experiment there was no correlation between the diversity of the ciliate populations in either temperature system and time, but that in the second experiment, there was a significant positive correlation between diversity and time for both ciliate populations ($P < 0.01$). These differences may be accounted for by the differing composition of the ciliate populations due to seasonal variation in the freshwater community.

The results obtained from both these experiments suggest that, in the conditions of these experiments at least, deterministic perturbation of an environmental factor leads to a

Fig. 2 Changes in temperature and diversity with time during expt 2. The lower graph shows the change in diversity ($\log_{10} \alpha$) with time (d) for the fluctuating-temperature system (○) and for the constant-temperature system (●). The upper graph shows the change in temperature (°C) with time (d) for the two temperature regimes, fluctuating temperature (○) and constant temperature (●).

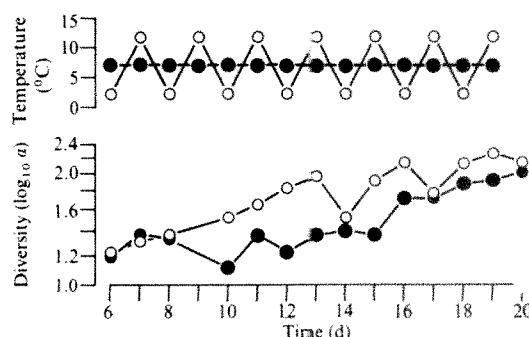


Table 1 Analysis of variance for experiment 1 giving the between-environment to within-environment variance ratio (F) for experiments 1 and 2

Source of variation	d.f.	Sums of squares	Variance s^2	F
Between environments	1	0.77	0.77	
Within environments	20	0.93	0.05	
Total	21	1.70		
Expt 1				15.4*
Expt 2				4.8†

* $P < 0.01$.† $P < 0.05$.

greater diversity of the ciliate population. The decreased index of diversity in the constant-temperature system is due to the increasing relative abundance of a small number of genera which dominate the ciliate populations. In the fluctuating-temperature system the relative abundances of the genera present are comparatively lower than those in the control system, and domination of the ciliate populations by a small number of genera does not occur to the same degree. It would seem that in the fluctuating-temperature system the environmental variations may be reducing the number of individuals present such that competition plays only a minor part in population control, thus leading to more even abundances of the genera present. In contrast to this, the constant-temperature system has allowed the part played by competition in population control to increase, such that a few genera have competitively excluded the others and so have become dominant in the ciliate population.

This reasoning is supported by Connell's assertion⁷ that the high species diversity of tropical rain forests and coral reefs is caused by infrequent catastrophic environmental perturbations which keep the community in a nonequilibrium-nonclimatic state, thus reducing the chance of elimination of less-able competitors, as is suggested by these experiments which use deterministic and less extreme perturbations.

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Attraction of the tick *Ixodes neitzi* to twigs marked by the klipspringer antelope

THE klipspringer (*Oreotragus oreotragus*), a small African antelope which lives in specific rocky outcrops, secretes a dark pungent resin-like substance from the ante-orbital glands which is utilised as a means of intraspecies communication¹. Marking is carried out by rubbing the ante-orbital glands on a substrate such as the twigs of shrubs on which the secretion is deposited. We have now observed that the adults of the tick *Ixodes* (*Afrimex*) *neitzi*² aggregate on twigs which have been previously marked by the klipspringer (Fig. 1). The attractive component of

the secretion is water soluble. The capability of the adult tick to detect the klipspringer scent was demonstrated in field observations and with laboratory experiments.

Adults of *I. neitzi* were collected from shrubs in the Rhodes Matopos National Park, Rhodesia. *Securinea virosa* was the dominant plant species in this area and grew from cracks in the rocks or closely to rocks.

The effect of the resin-like secretions collected from the twigs was assessed using the petri dish technique³ with minor modifications. Each 9-cm Petri dish was marked into eight sectors and because *I. neitzi* were very sensitive to desiccation, moistened discs of filter paper were placed in each sector. At the start of each experiment, 10 ticks (male or female) were placed in the centre of the petri dish. The tested materials were always placed in sector No. 8. A concentrated aqueous extraction of twigs marked by the klipspringers was the only substance which significantly attracted the ticks (Fig. 2).

Further examination using a Y-shaped olfactometer^{4,5} showed that none of the substances tested were attractive ($P < 0.5$, t -test for paired observations). The failure of the ticks to detect extract in the olfactometer and the fact that *I. neitzi* adults did not aggregate around freshly applied deposits which had been moistened, suggest that the scent secretion of the klipspringer is not airborne and only becomes attractive to the ticks or any other arthropods after a copious amount of water had been applied to the extract.

The solid secretion was extracted with different solvents and only the water soluble fraction was active in attracting the ticks (Fig. 3).



Fig. 1 a, Illustration of a twig marked by scent secretion from the ante-orbital gland of klipspringers. b, Assembly of *Ixodes neitzi* adults to a marked twig.

Uncontaminated branched twigs from the same shrubs from the experimental area were selected. The base and left arm of the Y-shaped twigs were painted with a concentrated aqueous solution prepared from marked twigs collected in the field. The Y-shaped twigs were positioned in soil with the two arms pointing upwards. Adult ticks were released on the soil and allowed to migrate. As the ticks moved up the vertical structure they were able to select one of the arms of the twigs.

These experiments were conducted in the field and repeated in the laboratory. In the field $66.2 \pm 2.9\%$ ($P < 0.001$) of the adults released in the vicinity of the Y-shaped twigs ascended the arm on which the aqueous klipspringer scent extract was applied. In the laboratory similar results were obtained where $63.7 \pm 1.00\%$ ($P < 0.001$) of the adults selected the treated arm. With similar but untreated Y-shaped twigs $52.2 \pm 2.08\%$ ($P < 0.3$) of the adult ticks selected the left arm.

In some *Ixodes* species an assembly pheromone has been shown to be secreted before feeding commences^{6,7}. To exclude any possibility of prior contamination of the marked twigs with a tick assembly pheromone, an aqueous extract was made from the ante-orbital glands of a klipspringer. When this material and

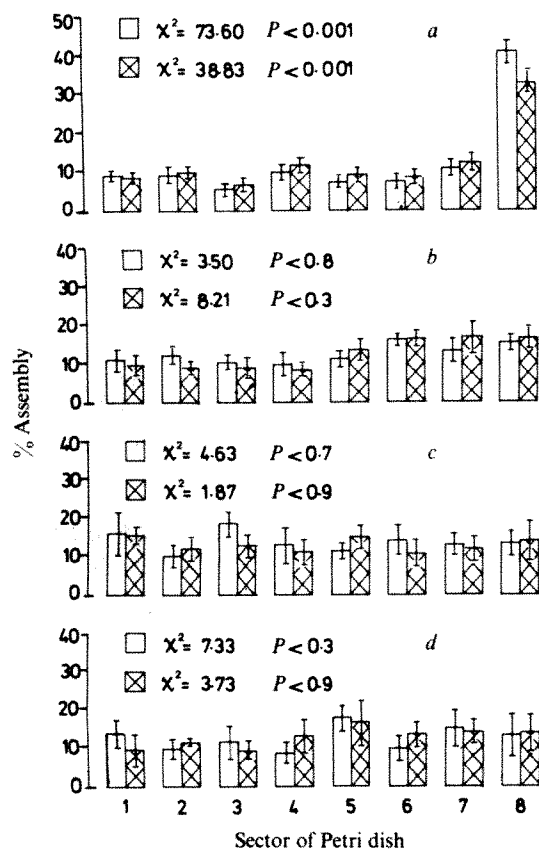
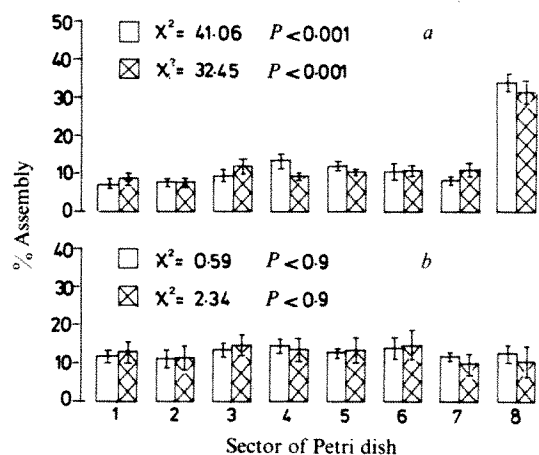


Fig. 2 Average assembly response (% $\pm$ s.e.) of *Ixodes neitzi* females ($\square$) and males ($\boxtimes$) to various materials placed in sector 8. *a*, Concentrated aquatic extract (30%) of scent marks of klipspringer. *b*, Dilute aquatic extract (10%) of scent marks of klipspringer. *c*, Scent marks of klipspringer which had been dipped in water before the experiment. *d*, Solution of unmarked twigs (control).

an aqueous extract of marked twigs were compared no differences were found, showing that the attractive principle arose from the klipspringer orbital gland secretions and excluding the possibility of pheromone contamination from the tick.

The klipspringer exudate apparently solidifies rapidly after being deposited on the tips of the twigs (Fig. 1a). This substance is subjected to the heavy rains falling between November and March (800 mm). The active component dissolves in the water, drips or runs down through the branches and stems to the

Fig. 3 Average assembly response (% $\pm$ s.e.) of *Ixodes neitzi* females ($\square$) and males ($\boxtimes$) to various materials placed in sector 8. *a*, Water soluble fraction of the scent marks of the klipspringer. *b*, Methanol soluble fraction of the scent marks of klipspringer.



ground level. We assume that the adult ticks, probably by using a gustatory sense, respond to the extract and follow it to the tip of the twigs. The adults remain inactive on the twigs until the return of the klipspringer to re-mark the area. Sniffing of the marked twigs by the klipspringer and consequent physical movement of the shrubs, stimulate the tick to gain access to the host.

This constitutes the first report of a tick species locating its mammalian host by detecting a specific chemical compound or compounds, used by the host as a communicative marking signal, thus increasing its probability of survival in that particular habitat.

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Mixed ligand chelate therapy for plutonium and cadmium poisoning

SERIOUS environmental and industrial hazards are associated with the release of radioactive¹⁻³ and nonradioactive⁴⁻⁷ metals. Most treatments for acute and chronic metal poisoning use chelating agents forming soluble, stable and excretable metal complexes¹⁻⁹. Although a few chelants are used clinically⁸, the efficacy of single-chelant therapy and variations is limited¹⁻³ and deleterious effects can occur^{4-7,9,10}. Consequently, Schubert recommended^{11,12} the use of mixed-ligand chelates (MLCs), in which the metal binds two or more different chelants, because of the enhanced stabilities attainable. For example, formation constants of thorium MLCs can exceed that of EDTA alone by at least 10^{13} (refs 13-15). We report here hitherto unparalleled achievements using MLC treatment, namely, complete removal of tissue deposits of ²³⁹Pu and prevention of mortality in animals given lethal, $>LD_{100}$, doses of cadmium.

Female, HA(ICR) mice, 45 d old, weighing 25.1 ± 0.6 g, were injected intravenously with plutonium or cadmium solutions; chelants were usually administered intraperitoneally. Monomeric¹⁶⁻¹⁸ ²³⁹Pu(IV) in 1% sodium citrate and cadmium chloride with and without addition of ¹⁰⁹Cd were used. Radiochemical assays¹⁹ were made from wet ashed tissues and excreta, and the activity measured in a liquid scintillation counter.

Table 1 Effect of single and mixed ligand chelate treatment on the tissue levels and total excretion of ^{239}Pu in female mice after Pu administration

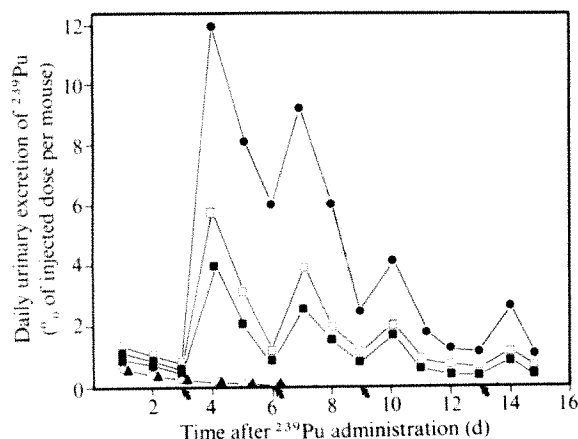
Sample	% Of injected dose of ^{239}Pu				
	Untreated controls	SA(2.0)	DTPA (0.5)	DTPA (2.5)	DTPA + SA (0.5 + 2.0)
Urine	4.3	5.4	24.8	28.4	54.0
Faeces	28.2	26.2	41.4	44.6	46.6
Liver	5.4 ± 0.3	5.1 ± 0.5	0.33 ± 0.1	0.22 ± 0.1	0 ± 0.01
Skeleton	33.7 ± 1.3	33.5 ± 2.1	13.8 ± 0.5	13.6 ± 0.4	0 ± 0.1
Total	71.6	70.2	80.3	86.8	100.6
Body (calculated)	67.5	68.4	33.8	27.0	0

The chelate treatment shown is in mmol per kg, and was given twice weekly. Measurements were taken 33 d after Pu (0.0162 μCi per mouse) administration and 30 d after the onset of treatment. The liver and bone levels represent average values $\pm$ s.d. from six mice, and the urine and faeces represent values per mouse from analyses on pooled samples from three mice. The total skeletal content was obtained by multiplying the amount of Pu in both femurs by 12 (refs 17, 18). The body values were calculated by subtracting the urine + faecal values from 100. See text and legends to Figs 1 and 2 for experimental details.

Polyaminopolycarboxylic acids (PAPCs) were administered as calcium-sodium salts to prevent hypocalcaemia, and components of MLCs were pre-mixed, that is, injected as a single solution.

Three days after Pu administration, when almost all had been deposited in the tissues^{16-18,20}, twice weekly injections of MLC [diethylenetriaminepentaacetic acid (DTPA) and salicylic acid (SA)] and of the individual chelants were initiated. Daily urinary excretion (Fig. 1) demonstrated that DTPA + SA increased Pu excretion to levels far above those induced by DTPA alone; SA alone had no effect (Fig. 1). By day 33 post-Pu, all injected Pu had been eliminated in urine and faeces (Table 1). After 10 treatments with massive doses of DTPA, skeletal Pu remained at 15% of the injected dose, whereas that of DTPA + SA-treated mice was, within counting statistics, free of Pu (Fig. 2 and Table 1). The livers of MLC-treated mice were devoid of Pu after four treatments. When treatment was delayed until 6 d post-Pu, 12 DTPA treatments reduced skeletal Pu to $24.3 \pm 0.3\%$, in agreement with previous work²¹, compared to $1.4 \pm 0.2\%$ of injected dose after 12 MLC treatments.

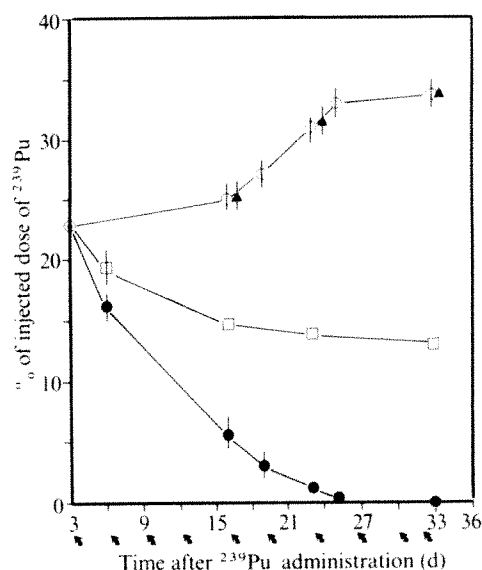
Fig. 1 Effect of single and mixed ligand chelates on the daily urinary excretion in female mice of plutonium-239 (specific activity $0.063 \mu\text{Ci} \mu\text{g}^{-1}$). The background count was 23.0 ± 0.5 d.p.m. Each point represents ^{239}Pu values per mouse from pooled samples of three mice. The animals were given a single intravenous injection of monomeric Pu(IV)-citrate of $0.0162 \mu\text{Ci} = 35,600$ d.p.m. The counting efficiency was almost 100%, as determined with calibrated standards. Three days later, treatment by intraperitoneal injection began and continued twice weekly. The DTPA was administered as the CaNa_3DTPA salt and that of SA as the sodium salt. The DTPA + SA were pre-mixed and injected as a single solution. ●, DTPA + SA (0.5 mmol per kg + 2.0 mmol per kg); □, DTPA (2.5 mmol per kg); ■, DTPA (0.5 mmol per kg); ▲, SA (2.0 mmol per kg); ○, Pu controls; arrows indicate treatment.



In experimental, acute Cd poisoning, DTPA or EDTA are marginally effective^{2,3,22-25}, and dimercaptopropanol is contraindicated^{4,8,25}. MLC treatment of mice 1 h post-Cd with EDTA + SA or EDTA + 2,3-dimercaptopropanol-1-sulphonate (Dimaval) (DMPS) resulted in 100% survival, whereas all single-chelant-treated animals died (Fig. 3). The therapeutic effect of the MLCs reflected a strong decorporative action. Thus, after a dose of ^{109}Cd -labelled chloride which was 4.5 times the LD_{100} , a single EDTA + SA injection 1 h later removed 82% of the Cd present in the kidneys at the onset of treatment; EDTA was ineffective.

The effectiveness of MLC therapy is maintained when low, clinically accepted doses¹⁻³ are used; for example, 0.05 mmol per kg for PAPCs and 0.2 mmol per kg for secondary chelants. The MLCs are also effective when administered orally. Many

Fig. 2 Effect of single and mixed ligand chelate treatment on the concentration of ^{239}Pu in the skeleton of female mice. Each point represents the average value of analyses made on the femurs of each of six mice. The skeletal values were obtained by multiplying the femur values by 12 (refs 17, 18). As the skeletal levels of ^{239}Pu for DTPA doses of either 0.5 mmol per kg or 2.5 mmol per kg were almost identical, one symbol was used for both. The SA dose was 2.0 mmol per kg, and the MLC was 0.5 mmol per kg DTPA + 2.0 mmol per kg SA. The error bars represent the standard deviation and are not included when the symbol diameter exceeds the magnitude of the standard deviation. Treatment with single or mixed ligand chelants began 3 d after the intravenous injection of $0.0162 \mu\text{Ci}$ of ^{239}Pu in the monomeric citrate form. Further experimental details are given in the text and legend to Fig. 1. ○, Pu controls; ▲, SA; □, DTPA; ●, DTPA + SA; arrows indicate treatment.



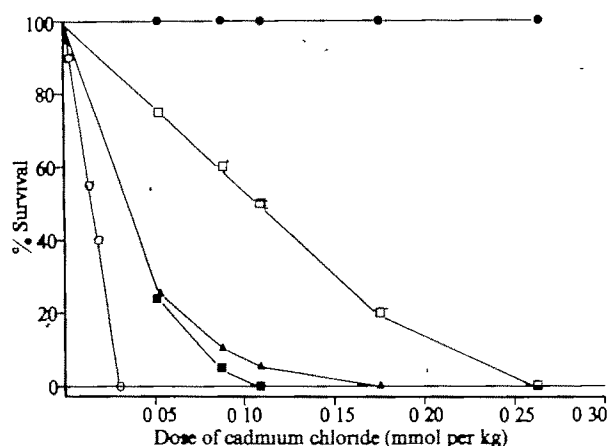


Fig. 3 Effect of treatment with single and mixed ligand chelates on the survival of female mice given increasing doses of cadmium. Each point represents the % survival of a group of at least 20 animals. The mice were given a single injection in the tail vein, of an aqueous solution of $\text{CdCl}_2 \cdot 2.5\text{H}_2\text{O}$ (MW 228.3). One hour later each mouse received a single, intraperitoneal injection of the specified chelant or mixed chelant solution. The untreated controls received a single i.p. injection of isotonic saline solution. Animals which survived 3 d remained healthy for the remainder of the observation period (4 weeks). ●, EDTA+SA or EDTA+DMPS; □, EDTA; ▲, SA; ■, DMPS; ○, Cd controls.

MLCs consisting of PAPCs with bidentate aromatic compounds or other bidentate compounds or PAPCs are at least as effective for Pu decorporation and therapy of other metals including Cu, Zn, La, Fe(III), Ni, Pb and Th, (these MLC systems include EDTA or DTPA combined with 1,2-diamino-cyclohexanetetraacetic acid, dithiocarb, hydroxyethylene-diaminetriacetic acid, nitrilotriacetic acid, penicillamine, salicylhydroxamic acid, sulphosalicylic acid and Tiron.) We are also investigating combinations of MLCs for decorporation of polymeric and insoluble forms of plutonium and other metals largely resistant to single-chelant treatment¹⁶⁻¹⁸ by using Fe-effective MLCs to facilitate decorporation of metals, for example, Pu, associated with endogenous iron species such as transferrin and ferritin.

Many chemical considerations play a part in the choice of MLC systems. For example, for 6-coordinate Cd(II), the MLC of EDTA+SA (total dentateness 8), as anticipated²⁶⁻²⁹, is more effective than DTPA+SA (total dentateness 10), as the presence of four unbound carboxyl groups in DTPA+SA, compared to two in EDTA+SA, is de-stabilising. The greater stability of many MLCs, larger than that predicted from statistical factors^{30,31}, derives^{13-15,26-31} from charge neutralisation, steric effects, intramolecular electrostatic interactions and cooperative electronic effects, particularly with aromatic secondary chelants. As the secondary chelant alone was largely inactive, we conclude that the primary metal chelate facilitates incorporation of the secondary chelant, presumably by deprotonation.

Experimental evidence of *in vitro* and *in vivo* formation of MLCs is provided by different chemical and biological approaches. When the total dentateness of the chelants was less or greater than the ligancy of the metal ion, for example, the metal chelates were hydrophilic, but they were lipophilic when the total dentateness was equal. Experimentally, we found that Pu(EDTA), Pu(SA) and the MLC of Pu(DTPA)SA were indeed hydrophilic, and that Pu(DTPA) and Pu(EDTA)SA were lipophilic as determined from partition coefficients using H_2O as the polar phase and *n*-heptane as the nonpolar phase. Ultrafiltration experiments^{11,12,32} on liver homogenates containing Pu or Th demonstrated greater effectiveness of MLCs relative to single chelants, and the superiority of EDTA+catechol (CAT) or DTPA+CAT relative to EDTA+SA or DTPA+SA, in agreement with potentiometric titration studies¹³.

In vivo MLC formation was demonstrated with yeast-fermented rats³³ in which varying concentrations of injected Zn(EDTA) or Cu(EDTA) reduced salicylate antipyresis to a predictable, quantitative degree³⁴. Removal of endogenous metals such as Zn by MLCs is similar to that by EDTA and DTPA (refs 2, 35-37), but treatment twice weekly, because of dietary replacement, produced no toxic symptoms. Toxicological and pharmacokinetic studies on MLCs using ^{14}C - and ^3H -labelled compounds are underway.

Mixed ligand chelate or complex formation by naturally occurring ligands and metals enters into biochemical processes and environmental situations, such as transport of metals from soils into the food chain and ground water. The ability of MLCs to remove all ^{239}Pu permits investigation of the mechanisms of tumour formation by Pu (refs 2, 3, 38) and modification of the genetic-toxicological effects of metals generally. The large variety of MLC systems available opens the way for major advances in the prophylaxis and treatment of metal toxicity, radioactive imaging with γ -emitting radiometals and diagnosis of exogenous metal body burdens by provocative or challenging doses⁴.

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Evidence that the TSH receptor acts as a mitogenic antigen in Graves' disease

GRAVES' DISEASE (GD) is an autoimmune disorder associated with the formation of thyroid antibodies, one class of which, thyroid-stimulating antibodies (TSAb), is thought to be involved in the pathogenesis¹⁻³. TSAb act similarly to thyrotropin (TSH) on the thyroid follicle cell through the mediation of adenylate cyclase⁴. The formation of TSAb is thought to be due to a change in the immune tolerance or to some alteration of the antigen, which has been suggested to be the TSH receptor². Some experimental evidence favours a defect in the immune surveillance, caused by decreased suppressor T-cell function^{2,5}. It has been reported that sensitised lymphocytes from patients with GD can produce TSAb *in vitro* after nonspecific stimulation^{6,7} as well as after specific stimulation with thyroid homogenate⁸. We report here that the lymphocyte-stimulating effect of thyroid membranes is abolished if the thyroid membranes are preincubated with TSH. According to our hypothesis, this means that the TSH receptor is the mitogenic antigen.

Eleven patients with GD and seven with toxic nodular goitre (TNG) were studied. The patients were hyperthyroid according to clinical and laboratory data (Table 1). The grouping of the patients into GD and TNG was based on clinical signs and on thyroid palpation and scintigraphy. Lymphocytes from these patients were studied with the lymphocyte transformation test. The method is described in the legend to Fig. 1. The stimulation index (SI) was calculated by dividing the mean c.p.m. of the three test cultures by the mean of the three control cultures. The mean lymphocyte SI for 10 healthy persons tested with thyroid membranes as the antigen was 1.02 ± 0.22 (mean $\pm$ s.d., range 0.61–1.35). The stimulation was considered positive when SI was more than 1.50, that is, mean + 2 s.d. In control incubations with leucoagglutinin (LA) as the mitogen, the SI varied from 20 to 90, reaching its maximum on day 3, and from 3 to 10 on day 7. SI with purified protein derivative of tuberculin (PPD) varied

Table 2 The effect of preincubation of LA and PPD with TSH

LA	Stimulation index with		
	LA + TSH	PPD	PPD + TSH
52.9	62.9	4.5	4.2
38.0	36.4	2.3	1.8
34.8	32.1	1.7	1.7
26.2	25.3	15.0	13.0
26.1	24.9		
32.0	36.8		

For further details of the lymphocyte transformation test and preincubations see the legend to Fig. 1.

from 1.7 to 15. The membranes from kidneys did not show any lymphocyte-stimulating effect in any patients tested. The coefficient of variation between triplicates was 4.1%.

When thyroid membranes were used as antigen, the mean SI was 3.64 in the patients with GD, nine of whom showed a positive response. A positive response was also seen in two patients with TNG (Table 1). Figure 1 shows the results for the 11 patients with a positive lymphocyte transformation test when leukocytes were incubated with thyroid membranes and with membranes which were preincubated with TSH. The lymphocyte stimulation disappeared completely in eight cases and partly in three. Thus, it seems that preincubation of the mitogenic membrane preparation with TSH blocked the mitogenic determinant. Preincubation of the thyroid membranes with human chorionic gonadotropin (hCG), which has the same α -subunit as TSH, failed to influence the stimulating effect of membranes (Table 1). Preincubation of LA or PPD with TSH did not substantially interfere with the mitogenic activity of either LA or PPD (Table 2).

The fact that preincubation with TSH inhibited the stimulating effect of the thyroid membranes on lymphocytes from patients with GD is considered strong evidence in favour of the hypothesis that autoimmunisation against the TSH receptor on the thyroid follicle cell membrane is the pathogenic mechanism of hyperthyroidism in Graves' disease. In other words, it seems

Table 1 Diagnosis and stimulation index in patients with Graves' disease or toxic nodular goitre

Patient	Sex	Age	Diagnosis	PBI (0.32–0.63 mol l ⁻¹)	T ₄ (50–135 nmol l ⁻¹)	FT ₄ (30–85 pmol l ⁻¹)	Lymphocyte transformation test		
							Stimulation index with		
							Thyroid membranes	Membranes preincubated with TSH	Membranes preincubated with hCG
L.B.	F	46	GD		253		2.16	1.40	2.20
A.L.	F	33	GD		250	450	1.77	1.00	1.70
H.T.	F	38	GD		246		0.40	0.47	
O.R.	M	34	GD		205		2.10	1.00	
L.R.	M	52	GD	1.06			3.00	1.86	
J.T.	F	36	GD		148	208	2.00	0.40	
F.B.	F	29	GD	1.25			1.00	0.40	
K.V.	M	66	GD		177		5.40	0.90	
S.P.	M	62	GD	0.72			11.10	0.60	
R.B.	F	41	GD		151		4.50	2.40	
K.A.	F	19	GD		280	158	6.55	1.60	
E.L.	F	34	TNG	0.54			1.70	0.90	1.70
V.T.	M	52	TNG		232		14.70	1.10	
F.A.	F	72	TNG		152	154	0.20	0.10	
J.P.	F	27	TNG		183	199	1.00	0.40	
B.J.	F	68	TNG		131	135	1.00	0.30	
P.M.	F	72	TNG	0.86			0.66	0.30	
E.V.	F	31	TNG	0.95			1.00	0.30	

The diagnosis of hyperthyroidism was based on clinical symptoms and signs as analysed by Lamberg *et al.*¹⁴. The diagnosis was confirmed by at least one of the following tests: serum protein-bound iodine (PBI), total thyroxine (T₄), and free thyroxine index (FT₄) as calculated from T₄ and triiodothyronine uptake by Sephadex (T₃U)¹⁵. The normal values are given in parentheses under the headings. The division into toxic Graves' disease (GD) and toxic nodular goitre (TNG) was made by palpation and thyroid scintigraphy. The lymphocyte transformation test was carried out within 2 months of diagnosis. For further details of the lymphocyte transformation test see legend to Fig. 1.

that lymphocytes of patients with GD are sensitised against the patients' own TSH receptor molecule, thus inducing TSAb production. TSAb obviously cause hyperthyroidism in most patients with GD. Some patients, however, do not develop hyperthyroidism, possibly because the functional capacity of the thyroid is impaired by coexisting destructive autoimmune processes^{9,10}. Another possible reason for the lack of response is that in some patients the TSAb are merely blocking antibodies¹¹, which do not stimulate the thyroid adenylate cyclase system.

The fact that the lymphocyte transformation test was positive in only two cases with TNG indicates that this type of hyperthyroidism is not usually due to a breakdown of the immune tolerance against the TSH receptor. However, nodular goitre

and GD may coexist, as shown in studies on a long-acting thyroid stimulator¹². In the present study, one of the two cases with TNG who had a positive SI was also tested by radioreceptor method for TSAb¹³: a positive TSAb index was found.

We do not know why the self-tolerance against TSH receptors is broken, nor why GD is self-limiting. The pathogenesis of TNG is unlikely to be related to the autoimmunisation phenomena seen in GD, but nodules may exist in a GD gland.

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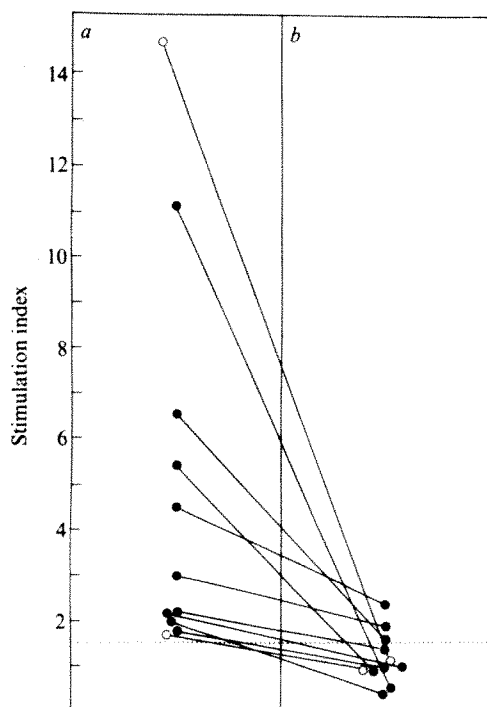


Fig. 1 The effect of preincubation of thyroid membranes with TSH on their stimulating ability on lymphocytes from nine patients with Graves' disease (●) and two patients with toxic nodular goitre (○). *a*, Stimulation index (SI) after incubation with thyroid membranes; *b*, SI of lymphocytes from the same patients after preincubation with TSH. 40 ml of blood was defibrinated with glass beads and mixed with 6 ml of a 6% dextran (MW 110,000) solution and allowed to sediment for 1 h at 37 °C. The leukocyte-rich plasma was collected and the leukocytes were washed three times in 5 ml Hanks' balanced salt solution and suspended in Eagle's minimal essential medium (MEM) containing 100 units ml⁻¹ penicillin, 100 µg ml⁻¹ streptomycin, 2 mM L-glutamine and 10% fetal calf serum (Flow). Triplicate cultures of 1 ml containing 2×10^6 leukocytes were incubated for 7 d with or without added antigen. After 6 d incubation at 37 °C in a humidified atmosphere (5% CO₂, 95% air) 1 µCi of ¹²⁵I-labelled deoxyuridine was added to each tube. 18 h later the cells were collected on to glassfibre filter papers using a multiple automated sample collector. The filters were dried at 60 °C and counted in a gamma counter. The antigen used was a thyroid membrane preparation (10,000g)¹³ which was formalinised according to the method of Ross *et al.*¹⁶. Control experiments showed that formalinisation of the membranes did not influence their ability to bind TSH specifically. The antigen was used at a final concentration of 50 µg protein per ml of medium. The thyroid antigen was also preincubated with 500 mU TSH (Actyron, Ferring) to block the TSH receptors on membranes. Incubation was carried out for 20 min at 20 °C and used at the same concentration as the thyroid membranes alone. The control experiments with LA (Pharmacia) and PPD (State Serum Institute, Copenhagen) and the preincubations with TSH were carried out according to the same culture method. The LA concentration used was 5 µg ml⁻¹, PPD 250 IU ml⁻¹, hCG 50 IU and TSH 500 mU.

Graft-versus-host disease impairs cerebellar growth

THE mechanisms of mental retardation are of critical interest to both basic developmental neurobiologists and those in the clinical community concerned with perinatal development. The possibility of a deleterious immunological interaction between mother and fetus has long been recognised as a potential developmental hazard¹. However there have been no reports of specific conditions which involve immunological injury to the developing brain. Graft-versus-host disease (GVHD) is a model disease that can be induced naturally or experimentally by grafting mature, immunocompetent lymphoid cells into an immature, immunoincompetent host. The failure of the host, for example a newborn rat pup, to recognise the graft as non-self leads to an attack by the grafted cells on host lymphomyeloid tissues, resulting in many symptoms, including wasting, anaemia, alterations of lymphoid organ weights, hyperexcitability and an ataxic-like gait². The last two features suggest possible brain dysfunctions and have motivated our investigation of immunologically induced perinatal brain injury. The rat cerebellum was used here as a model system for the study of brain alterations during GVHD because of its well known cytoarchitecture³. In addition, its postnatal development renders it sensitive to many forms of perinatal stress^{4,5}. In this

Table 1 Effect of graft-versus-host disease on several cerebellar growth parameters

Day killed	Injected cells	Cerebellar weight (mg)	Cerebellar contents (mg)			
			DNA	RNA	Protein	DNAF/ASF
GVHD-producing LNC injected						
9	None	63.3 ± 1.7	0.45 ± 0.02	0.37 ± 0.01	4.4 ± 0.1	0.43 ± 0.03
	FLNC	61.1 ± 1.2	0.48 ± 0.01	0.38 ± 0.01	4.4 ± 0.1	0.46 ± 0.04
11	None	85.4 ± 1.2	0.65 ± 0.02	0.44 ± 0.01	6.7 ± 0.3	0.81 ± 0.04
	FLNC	75.3 ± 1.4*	0.54 ± 0.02*	0.39 ± 0.01*	6.1 ± 0.3*	0.53 ± 0.02*
13	None	110.9 ± 1.7	0.89 ± 0.03	0.54 ± 0.01	7.2 ± 0.1	0.54 ± 0.02
	FLNC	102.6 ± 1.8*	0.77 ± 0.01*	0.47 ± 0.02*	6.4 ± 0.2*	0.35 ± 0.03*
14	None	119.0 ± 2.1	0.95 ± 0.03	0.55 ± 0.02	9.0 ± 0.2	0.76 ± 0.05
	FLNC	82.2 ± 4.2*	0.73 ± 0.07*	0.43 ± 0.04*	7.2 ± 0.4*	0.34 ± 0.03*
Non-GVHD-producing LNC injected						
14	None	121.4 ± 4.8	1.04 ± 0.06	0.60 ± 0.02	8.6 ± 0.6	0.62 ± 0.03
	F ₁ hybrid					
	LNC	124.4 ± 4.4	1.05 ± 0.04	0.58 ± 0.02	8.6 ± 0.4	0.58 ± 0.04

Animals injected with either 40×10^6 Fischer lymph node cells (FLNC) or F₁ hybrid LNC were compared with non-injected littermates. DNAF/ASF is the ratio of c.p.m. per g in the DNA fraction to c.p.m. per g in the acid-soluble fraction 1 h after an i.p. injection of ^3H -TdR (2 μCi per g body weight). Results are expressed as mean $\pm$ s.e.m.

* Significantly less than non-injected controls (None), $P < 0.05$.

study, we demonstrate that significant alterations in the cerebellar cellular content and capacity for DNA synthesis occur in rats suffering from neonatally induced GVHD.

GVHD was induced by injecting 40×10^6 parental strain lymph-node cells (PSLNC) on the day of birth (day 0) into the F₁ hybrid offspring of two inbred strains of rats (Fischer and DA). Newborn F₁ hybrid animals were used because they are genetically tolerant of grafts from either parent and, thus, especially susceptible to immunological attack by parental lymphocytes. Non-injected littermates or littermates injected with harmless, syngeneic F₁ hybrid LNC were used as controls. At various ages, the pups were killed and alterations in cerebellar development assessed. First, cell acquisition was estimated by measuring total cerebellar DNA content. Second, the uptake of ^3H -thymidine (^3H -TdR) into the DNA tissue fraction from the acid-soluble tissue fraction was assessed 1 h after an intraperitoneal injection of 2 μCi per g body weight. General growth of the cerebellum was measured by determining organ weight changes and total organ RNA and protein content.

The time course of GVHD effects on cerebellar maturation is characterised by a rapid appearance of changes. Thus, at the ninth postnatal day, injected animals were relatively normal by the parameters measured here. However, at postnatal day 11 cerebellar development was noticeably altered.

As seen in Table 1, cerebellar weights were affected in animals injected with PSLNC. Between days 9 and 11 there was only a 19% increase in cerebellar weight in animals with GVHD, compared with a 26% increase in controls. Subsequent to this initial change in cerebellar weight gain, cerebella from GVHD animals remained significantly smaller than those of non-injected littermates. In addition to our assessment of GVHD effects on cerebellar weights we also weighed each intact animal, its spleen and thymus, and found weight alterations similar to those previously described in animals with neonatal GVHD⁶.

In animals injected with PSLNC, both cell proliferation and RNA and protein contents were decreased compared to either non-injected controls or controls injected with syngeneic cells. This effect was proportional to changes in cerebellar weight (Table 1).

At each day including and after day 11, there was significantly less DNA, RNA and protein, indicating a marked decrease in accretion of cell number and cellular content (Table 1). There was actually less DNA at day 14 than day 13 in cerebella from GVHD animals, possibly reflecting a degree of cell death in addition to the slowed cell proliferation. Compared to controls, the uptake of ^3H -TdR into the DNA fraction from the acid-soluble fraction was less in GVHD animals at each day

measured except day 9. Labelling of the acid-soluble fraction was not significantly different in GVHD compared to controls, but there was a great difference ($P < 0.001$) in the amount of label in the DNA fraction, suggesting that although cerebellar cells in GVHD animals can take up ^3H -TdR, the mechanism for incorporating the label into DNA is altered. It is especially noteworthy that these actions on brain growth occur at a time before body wasting is evident. Animals which received syngeneic cells, that is, lymphocytes not reactive against the host, were similar to non-injected controls with respect to either cellular acquisition or cellular content.

Histological preparations showed the regular arrangement of the layers of the cerebellum to be undisturbed in 14-d-old animals with the classic symptoms of GVHD. In addition, there was no mononuclear cellular infiltration of the cerebellum, which argues against a direct, cell-mediated attack by invading lymphocytes against cerebellar cells.

The demonstrated effects of GVHD on maturing rat cerebellum may be highly significant with respect to the development of the human cerebellum, which acquires 80% of its total neuronal cell population, that is, granule cells, during the first two postnatal years⁷. Therapeutic measures, including exchange transfusion of the immunologically immature fetus *in utero*⁸, routine transfusion of infants suffering from congenital immunological deficiency syndrome⁹, and even of lymphocytes from the maternal to the infant circulatory system¹⁰ can all place infants at risk due to the induction of a GVHD syndrome. In addition, bone marrow transplantation in children with haematological disorders can result in GVHD, and affected infants often survive mild or moderate episodes of the disease¹¹. Human infants do, in fact, receive foreign lymphocytes in these instances at a time when cerebellar cells are still proliferating, but there has been no assessment of potential CNS damage due to induction of graft-versus-host disease.

The adverse actions described here of GVHD on cell proliferation in the developing rat cerebellum indicate that the CNS is a previously unrecognised site of damage during this syndrome. The mechanism by which GVHD causes damage to cerebellar growth is still obscure. No lymphocytic invasion of the cerebellar tissue was observed in affected animals, which suggests that a blood-borne factor may be involved. Alterations in cerebellar synthetic capacity could be the result of several different agents during the course of the disease; for example, hormones like cortisone¹² or factors from activated lymphocytes such as proliferation inhibitory factor.¹³ It is conceivable that the cerebellum could be affected as an innocent bystander in a similar way to other tissues such as the skin¹⁴ and intestine¹⁵ of

animals with GVHD. Our preliminary histological studies have suggested a depletion of cells in the external germinal layer, an effect quite different from that found in malnutrition¹⁶ and some hormonal alterations¹⁷. The analysis of the process by which graft-versus-host disease selectively slows growth within the proliferative cell population in the cerebellum remains a challenge to the understanding of basic growth processes in neural development, and is under investigation in our laboratory.

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Dissociation between learning and remembering in organic amnesia

IN Korsakoff's disease and certain other cases of organic amnesia there may be profound disturbance of memory with little or no other intellectual disturbance. The nature of this memory defect is controversial and in particular there is disagreement about the stage of the memory process which is impaired. There is evidence in support of three possibilities¹⁻³. Information might be inadequately acquired, maintenance of information in store might be defective or information might be inadequately retrieved from store. The results reported here suggest that inadequate acquisition is the most likely cause of the amnesia in Korsakoff's disease.

It is known that amnesic patients are inferior to controls in recognising previously seen pictures when conditions of presentation and testing are identical for both groups^{4,5}. However, when pictures are presented to Korsakoff patients for a period four to eight times longer than that given to controls, and both groups are tested 20 min later, the recognition performance of the patients is raised to that of controls⁶. We have used this effect to establish equal performance of two such groups on picture recognition after a 10 min interval and then to compare their speed of forgetting as shown by their performance after longer intervals. This obviates the difficulties that have beset previous attempts to compare the rates of forgetting of amnesic and control subjects (see ref. 7 for review).

The Korsakoff patients were seven severely amnesic males with a history of alcoholism. Four were tested at the Boston or Bedford Veterans Administration Hospitals (Massachusetts) and three were tested at the Manchester Royal Infirmary. All had a severe defect of recent memory but other cognitive

functions were intact. Their mean age was 58.4 yr and mean IQ on the WAIS scale was 104. The control group consisted of six males with no evidence of neurological or psychiatric disturbance who were ambulant inpatients on the general medical and surgical wards of the Boston VA Hospital. Their mean age was 58.8 yr and mean IQ 116.

Each subject was shown 120 slides of coloured pictures photographed from magazines (stimulus items) and asked to remember them. Recognition memory was tested 10 min, 1 d and 7 d later. In each test, a sample of 40 of the original pictures was presented, randomly interspersed with 40 new pictures (distractor items), and the subject was asked to say for each picture whether or not he had seen it previously. We aimed for 75% correct recognition by both groups after the 10 min interval. Because there was no certainty that individual subjects would achieve this level of performance after a particular exposure to the stimulus items, two sets of 240 pictures were prepared. These were all drawn from the same source and individual pictures were allocated randomly to the first or second set and, within sets, to stimulus and distractor items. The 120 stimulus items from the first set were shown to the controls for 1 s each and the range of correct responses 10 min later was 71.3-85%. The same stimulus items were presented for 4 s each to the Korsakoff patients. This enabled four of them to attain a level of performance after 10 min comparable to that of controls (78.8-83% correct). For the remaining three patients, 4 s was inadequate: their performance at this exposure was therefore treated as a calibrating experiment and was not analysed. Six hours later, these three patients were shown the second set of 120 stimulus items for 8 s each. This resulted in recognition after 10 min equal to that of the controls (72.5-80% correct). A Mann-Whitney 'U' test then confirmed that after 10 min there was no significant difference between the performance of the seven amnesic and the six control subjects ($P = 0.53$).

All subjects were tested again 1 and 7 d later, using slides from the appropriate set. The results are shown in Fig. 1. A two-way analysis of variance was carried out and this showed that the interval between exposure to stimulus items and testing (the retention interval) was the only significant source of variation ($F = 46.5$, $P < 0.001$). Neither the between-groups component nor the interaction between group and retention interval was significant. An identical pattern of results was obtained when the raw data and two kinds of transform of the data (inverse sine and logistic) were analysed. Detailed comparisons showed no significant difference between the groups for any of the three retention intervals. Furthermore, the distribution of true positive and true negative responses was virtually identical for the two groups for each retention interval (Table 1). Thus, recognition performance declines with increasing retention interval but the rate of decline does not differ between the groups. This identity of performance between the groups cannot be explained

Fig. 1 Mean % correct (TP+TN) responses (± 1 s.d.) on picture recognition test. Speed of forgetting is identical when initial performance (at 10 min) has been equated. Solid line, Korsakoff patients; dashed line, control subjects.

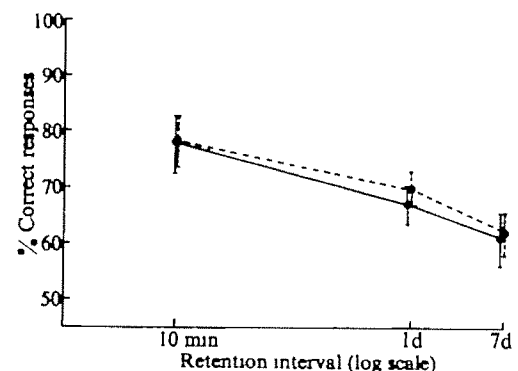


Table 1 Mean true positive (TP) and true negative (TN) responses on picture recognition test (maximum = 40)

	Retention interval					
	10 min		1 d		7 d	
	TP	TN	TP	TN	TP	TN
Korsakoff patients	26.7	35.6	21.1	32.0	19.4	29.4
Control subjects	28.5	34.2	24.2	31.6	20.5	29.2

by the somewhat different intelligence of the two groups for two reasons. (1) When Korsakoff patients and controls are strictly matched for IQ, their recognition performance is identical after a standard retention interval if the patients receive the stimulus for 4–8 times as long as do controls⁶. (2) In our experiment, the Korsakoff patients were on average somewhat less intelligent than the controls and therefore could not have compensated for amnesia with more intelligent strategies for learning and remembering. The performance of the Korsakoff patients was indistinguishable from that of the controls not only after 10 min (when performance of the two had been deliberately equalised) but also after the two longer intervals when any difference in the ability to remember should have become apparent. Clearly, it is not in their recognition performance that the groups differed, but in the time available to acquire information when the pictures were first presented.

The simplest interpretation of our results is that the amnesic defect of the Korsakoff patients resides in the initial learning of the information. This interpretation can be avoided only by assuming that the additional time for learning made available to Korsakoff patients resulted in their acquiring more information than the controls and losing this advantage within 10 min through defective retention or retrieval. However, such an advantage could not have been lost through faulty retention because this would require the curves to diverge after the 10 min test (Fig. 1) and the Korsakoff patients to perform abnormally poorly after the longer intervals. Nor could such an advantage be lost through greater susceptibility to interference from earlier memories—the most frequently advocated ‘retrieval theory’ of amnesia^{3,8–10}—such interference increases with retention interval¹¹ and accordingly the Korsakoff patients should forget faster than the controls. Again the curves should diverge.

It therefore seems very likely that the extra time provided for the Korsakoff patients was utilised to achieve a level of learning comparable with that which the controls achieved in less time. Accordingly, at least for picture recognition, the Korsakoff patients seem to have a defect in the initial learning of information but forget no faster than normal. Normal rates of forgetting in Korsakoff patients might seem paradoxical if organic amnesia is seen as inability to remember. However, apparent defects of retention or retrieval in these patients could in principle be—and, we suggest, are—secondary to a defect of initial learning.

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Mechanism for the formation of synaptic projections in the arthropod visual system

THE axons of photoreceptors in arthropods project in a precisely ordered manner from the retina to the lamina. As retina and lamina consist of two-dimensional arrays of structural units (the ommatidia and the cartridges, respectively), a particular unit of origin and a specific target unit can be defined uniquely for each axon. In a number of species, such as water fleas¹, crabs² and locusts³, all axons originating from an ommatidium terminate in the same cartridge, where they form synapses onto laminar neurones. How is a particular target cartridge selected by the reticular axons as they grow into the laminar anlage during embryogenesis? The adult pattern of projections could arise in several ways. For example, each ommatidium and each cartridge could have unique chemical labels varying with or according to their positions in their respective domains. Input and target could then be matched by a chemoaffinity mechanism such as that originally proposed by Sperry⁴ for the formation of vertebrate retinotectal connections. A second possibility is that reticular fibres do not recognise any specific labels in the laminar cells, but grow into this region in the appropriate spatio-temporal order to sequentially fill available sites in a pre-formed laminar array. In a variation of this second mechanism the lamina would be unstructured at the time of arrival of reticular axons and the axons would impose a pattern on the lamina by sequentially recruiting immature laminar neurones to form the regular array of cartridges. These possibilities have been tested by deleting ommatidia at specified developmental stages in *Daphnia magna*, a small branchiopod crustacean with a total of 22 ommatidia and 22 cartridges¹. The observations reported here support the last hypothesis, that the order of reticular axon ingrowth determines the retino-laminar projections in this organism.

The formation of the retino-laminar projection in *Daphnia* follows a precise sequence of events which occurs during the middle stages of embryogenesis^{5,6}. This is shown schematically in Fig. 1. Reticular axonal bundles arrive in order at the midline of the laminar anlage, where they contact immature laminar neurones with which they will subsequently form cartridges and move away from the midline. In order to determine whether a bundle of reticular fibres only interacts with a specific, pre-determined group of cells, I used a UV radiation microbeam to delete ommatidia during development before their axons made such contacts. Two possible outcomes of such an experiment are shown schematically in Fig. 2. On the left, cartridges which normally correspond to the deleted ommatidia receive no reticular projections; on the right, the last cartridges that would form are the affected ones.

Lesions were made in about 40 embryos at different locations in the eye anlage, deleting 1–10 ommatidia before their fibres grew into the lamina⁷. Animals were fixed at various times after the irradiation and serially sectioned for light or electron microscopy. The analysis was in part performed with the aid of three-dimensional computer reconstructions from these sections⁸.

Some data on experimental animals analysed as adults are presented in Table 1. For every ommatidium which was completely removed in the embryo, five laminar neurones, and

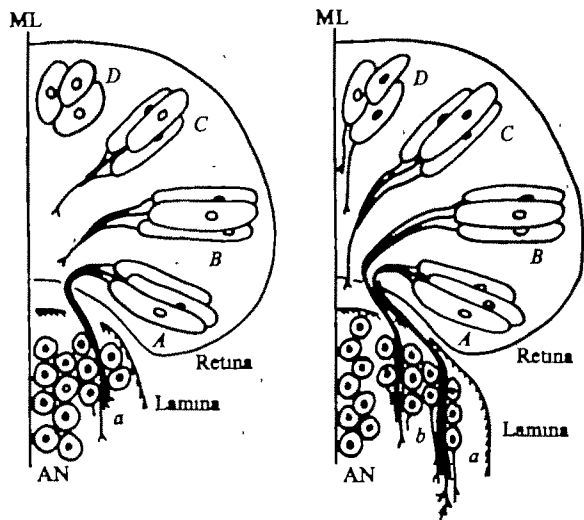


Fig. 1 Schematic diagram of a rostro-caudal cross-section of two *Daphnia* embryos; left, 30 h old; right, 33 h old. Only the eye and lamina on the right of the midline are shown. A–D, Ommatidia; a, b, cartridges. ML, midline; AN, available immature neurones. The photoreceptors in ommatidium A are the first to differentiate. They send processes along the midline into the lamina, where they encounter laminar cells without processes. Within each future ommatidium one cell matures earlier than the other seven; its growing fibre precedes or ‘leads’ the other seven into the lamina. The lead fibre sequentially contacts five laminar cells which then wrap around this fibre and form transient gap junctions with it^{5,6}. The cells which are contacted are always those closest to the anterior surface and the midline of the laminar anlage. As the following fibres of the bundle arrive, the laminar cells unwrap partially, form a ring around the bundle and elaborate processes which grow in parallel with the receptor fibres into the forming neuropil. After cartridge a is formed in the lamina, it moves laterally as the fibres from ommatidium B arrive. After cartridge b is formed, it also moves laterally behind cartridge a. The fibres from ommatidium C then arrive at the midline and contact five immature neurones. At 33 h, the first cartridges to form have begun to elaborate processes which grow into the future neuropil region. Synapses will appear a few hours later.

hence a cartridge, were missing in the adult. When an ommatidium was only partially deleted, however, a smaller than normal cartridge was formed. From these data it was not possible to determine conclusively which one of the cartridges was missing when an ommatidium was removed. In no case out of 17 analysed in detail were there found any areas filled by glia but devoid of neurones, or any groups of degenerating laminar neurones that would allow identification of the missing cartridges. In each case the cross-sectional area of the lamina was reduced in proportion to the number of missing structures. (It has also been shown that eye deletions strongly affect laminar structure by genetic manipulation in *Drosophila*⁹ and surgical manipulation in other species^{10,11}.)

Table 1 Numbers of reticular neurones, ommatidia, laminar neurones and cartridges in animals irradiated as early embryos and analysed as adults using 3-D computer reconstructions from serial sections

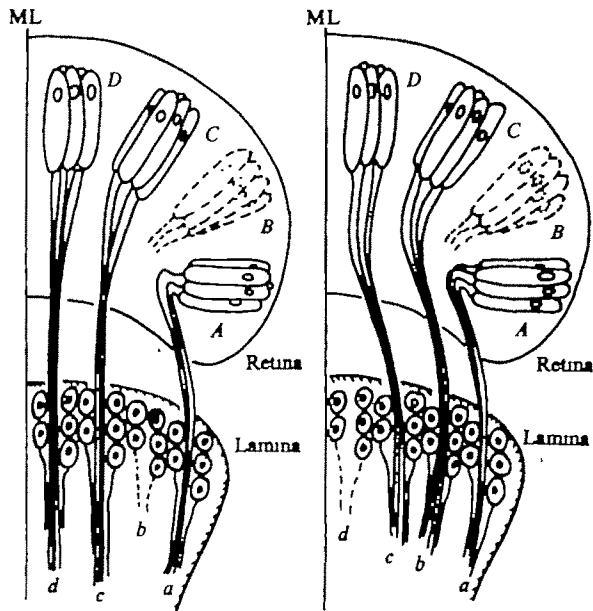
Specimen	No. of reticular neurones	No. of ommatidia*	No. of laminar neurones	No. of cartridges*
A	163	20	99	20
B	153	20	94	20
C	116	18	87	18
D	87	14	68	14
E	89	12	57	12
F	127	16	79	16
Control	176	22	110	22

* Some ommatidia and their associated cartridges have fewer than the normal complement of neurones (8 and 5, respectively) in experimental animals

Two observations on experimental animals analysed at embryonic stages ranging from 3 to 20 h after irradiation showed that the cartridges which degenerate are the last ones to form. First, immature laminar neurones, including those that later disappear, are still to be found within the laminar anlage until some hours after all reticular fibres normally arrive at the lamina. No signs of laminar cell degeneration are found until some 10–12 h after the 38 h stage, when the last bundles grow into the lamina (see Fig. 3). Second, no group of laminar neurones without an associated bundle of reticular fibres is found away from the midline at any stage during or after the arrival of the reticular bundles. In other words, at no developmental time is there a situation like that shown in Fig. 2 (left). Instead, laminar cells that are not contacted because of the reduced number of reticular bundles are found near the midline, as diagrammed in Fig. 2 (right) (see also Fig. 3). These left-over cells fail to elaborate fibres at the time when corresponding normal groups, located symmetrically about the midline, are well differentiated.

The extra laminar cells could, in principle, be either the last group to appear, group d in Fig. 2, or group b which, as it was never contacted because ommatidium B was deleted, has remained near the midline. My observations of the temporal pattern of fibre ingrowth and of the geometry of the laminar anlage support the first alternative. The immature neurones contacted by a growing fibre bundle are always those closest to the anterior surface and the midline. Therefore, if growing bundle C is to contact laminar group c, then group b must be displaced away from the midline. Yet a group of laminar cells is never observed away from the midline without an associated reticular fibre bundle. It is conceivable, though rather unlikely because of the geometry of the developing lamina, that groups b and c permute positions if b is not contacted at a specific time. However, in no experimental animal studied at the appropriate embryonic stage was there any indication that laminar cells move from the anterior surface back toward the more posterior regions from which they originally arise. Though I cannot absolutely dismiss the possibility that group b recycles back to

Fig. 2 Two possible outcomes of the deletion of ommatidium B. (Nomenclature as in Fig. 1.) Left, if ommatidium C recognises and contacts only the laminar cells which will form cartridge c, then we should find, at some developmental stage, a group of neurones in the b position which do not receive a reticular projection. Right, if the fibres from ommatidium C recruit whatever laminar cells are available, they will contact those cells that will normally become cartridge b. The deletion of ommatidium B then would lead to the d laminar cells remaining untouched and available.



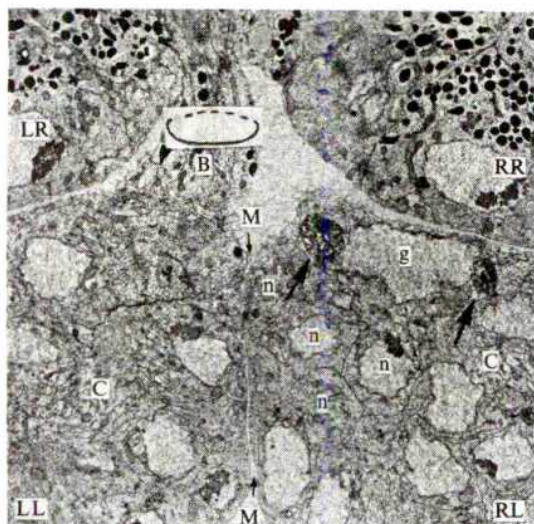


Fig. 3 Low power electron micrograph of a rostro-caudal section from a set of serial sections of an embryo irradiated on the right side at 31 h and fixed at 50 h. M, midline; LR and RR, left and right retina; LL and RL, left and right lamina; C, cartridge; B, fibre bundle travelling from LR to LL. The corresponding fibre bundle on the right was deleted by irradiation. Arrows indicate debris from degenerating cells which has been internalised by glial cell g. Immature neurones, n, that remain without an associated reticular bundle are found at this stage only on the right side of the midline. Computer reconstructions from the serial micrographs show that these immature neurones have failed to elaborate processes, unlike the neurones in corresponding positions on the left side of the midline. Note that, because of these left-over cells, cartridge C on the right is more distant from the midline than the one on the left. $\times 2,400$.

the surface region where the left-over cells are found, I believe from my observation of the temporal sequence of events that it is highly unlikely. I conclude that the extra cells are the last to arrive, that is, those that normally would have become part of the cartridge d.

From these observations the following developmental scheme in the *Daphnia* visual system can be formulated. As reticular fibres arrive at the laminar anlage they recruit whatever immature laminar neurones are available at that time and in that position. (This recruitment process could be mediated by signals exchanged during the early, characteristic interaction between reticular fibres and immature neurones which have been described previously⁶.) The order of synaptic projections is a consequence of the spatio-temporal sequence of ingrowth of optic fibres and no special affinity of ommatidia for specific targets is required. In animals with retinal lesions the extra laminar cells fail to differentiate because they do not receive a required signal from reticular fibres. The degeneration of laminar cells deprived of contacts provides evidence supporting cell death as the mechanism for regulating relative numbers of neurones in this system. Such a regulatory role for cell death has been previously suggested in both invertebrate¹² and vertebrate nervous systems¹³.

In conclusion, experimental evidence has been obtained which indicates that, in a structure whose parts normally mature in a spatially and temporally ordered fashion, neurones and their targets need not be individually labelled in order for appropriate synaptic projections to be achieved. Horridge³ arrived at a similar conclusion when he observed normal optokinetic responses by locusts that had regenerated eye-lamina connections following eye rotation at the second instar larval stage. However, as about two-thirds of the retina is added at later instars¹¹, it is unclear whether this result is due to normal development of the remaining retina following the graft rotation or to the regeneration of connections from the existing ommatidia. A spatio-temporal mechanism has also been suggested by Gottlieb and Cowan¹⁴ as a possible way to account for the

genesis of the pattern of synaptic connections in the dentate gyrus of the rat. In those cases where all fibres grow in and form synapses simultaneously, however, a different mechanism, such as chemoaffinity⁴ or fibre-sorting¹⁵, might be necessary.

Although sequential differentiation of photoreceptors also takes place in *Drosophila* and other insects (see ref. 16 for review), the details of the developmental interactions are not known in these species. My results on *Daphnia*, and parallels in the developmental process in various species¹⁶, suggest that mechanisms involving cell-cell contact as the signal for specific recruitment and differentiation of target cells may be generally valid in the formation of the retino-laminar system of invertebrates.

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Phase-locking in goldfish saccular nerve fibres accounts for frequency discrimination capacities

THE auditory systems of fishes have been of particular interest in the development of auditory theory because a mechanical analysis of frequency had seemed unlikely on the basis of the ear's rather undifferentiated structural organisation¹. Thus, at the level of the 8th nerve, information about stimulus frequency is thought to be coded in the temporal structure of neural activity rather than in across-fibre distributions of impulse rate²⁻⁴. However, recent neurophysiological data show that a limited peripheral frequency analysis occurs in several species⁵⁻⁷ and some workers have suggested that this may form the basis for psychophysical demonstrations of filtering⁸⁻¹⁰ and for frequency discrimination in the fish auditory system^{11,12}. Such a 'place principle' of frequency analysis and pitch perception is presently generally believed to originate in the phyletic series among the amphibia and to reach the greatest degree of elaboration with the mammals¹³. We report here an experiment which helps to resolve this question of frequency codings by evaluating the adequacy of the temporal hypothesis to account for the behaviourally measured frequency difference limens for the goldfish^{11,12}. A simple form of the temporal coding hypothesis states that the reciprocal of stimulus frequency (period) is

represented in 8th nerve fibres as the distribution of time intervals between phase-locked impulses⁴. A discrimination between two different period lengths may thus entail a decision as to whether the two resulting distributions of neural interspike intervals are the same or different. Clearly, the performance on such a discrimination would be limited by the variability of the distributions, or the accuracy with which neural impulses are phase-locked to the stimulating waveform. We demonstrate here that the neurones which phase-lock best at any given frequency show degrees of phase-locking accuracy that would be expected if the central nervous system were processing the input optimally.

The details of the method used for the neurophysiological measurements have been published previously⁷. Phase-locking variability of single saccular neurones of the goldfish was measured in response to tones presented at the frequencies and amplitudes used in behavioural measurements of frequency discrimination¹¹. Extracellular potentials were recorded from single saccular nerve fibres in 15-cm common goldfish, anaesthetised (MS-222) and immobilised (Flaxedil). Generally, saccular neurones could be classified into high frequency (HF) and low frequency (LF) types. The LF neurones showed best frequencies below 200 Hz, whereas the HF type showed best frequencies ranging between 300 and 800 Hz. Highly spontaneously active neurones showed little adaptation and were stimulated with continuous tones of up to 10 s duration. Neurones with little or no spontaneous activity showed rapid adaptation and were stimulated with 100-ms tone bursts, repeated at 2 Hz.

Neurones could be 'held' from seconds to over an hour, with the LF type usually lasting longer and exhibiting larger spikes (5–40 mV) than the HF type (0.5–20 mV). When a neurone was encountered, it was stimulated with a set of frequencies at sound pressure levels 35 dB above behavioural threshold¹⁴ until it was 'lost' or until the stimulus set was exhausted. For each signal, a 10-min period histogram¹⁵ was obtained from which measures of phase-locking variability were made. Figure 1 shows the period histograms obtained for four representative neurones

Fig. 1 Period histograms for four representative neurones, 204(a), 205(b), 209(c) and 216(d), from one animal for 1 s of sinusoidal stimulation at the frequencies indicated. Sound pressure levels are 35 dB above behavioural threshold¹⁴. The number associated with each histogram is the standard deviation of the distribution in ms.

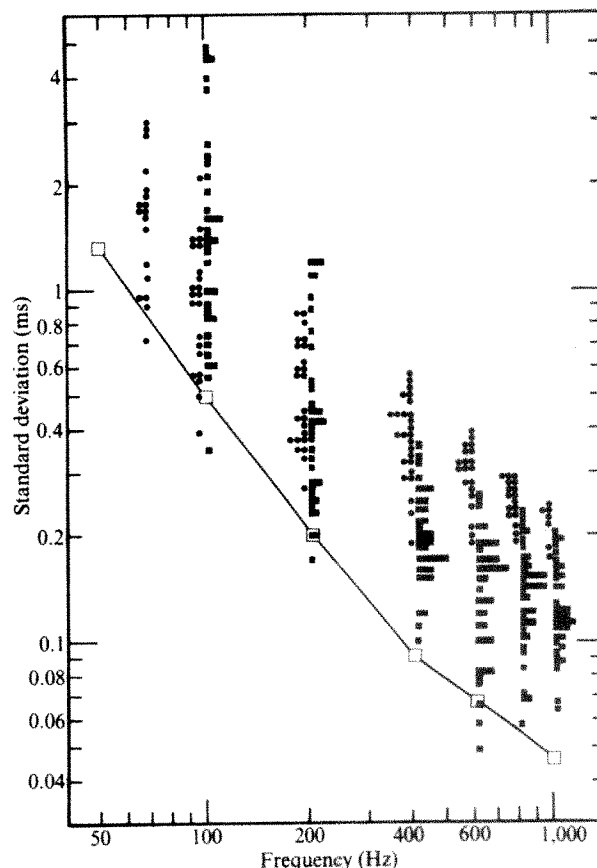
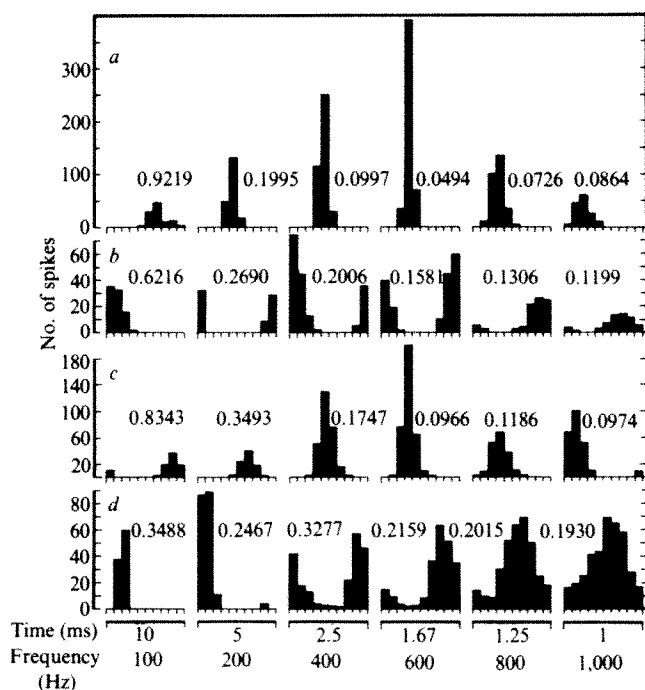


Fig. 2 The standard deviations (in ms) of period histograms for 45 high-frequency neurones (■) and 22 low-frequency neurones (●) to sinusoidal stimulation at 35 dB above behavioural threshold¹⁴ from nine animals. The line connects the mean just-noticeable differences in stimulus period (in ms) measured psychophysically for four animals in a previous study¹¹.

from a single animal. From each histogram, average discharge rate, the location of the mean (phase angle) and the coefficient of synchronisation (R) were calculated¹⁶. The coefficient R was then transformed into a standard deviation value in ms (ref. 17). These values are plotted as a function of frequency in Fig. 2 for 63 of the most sensitive neurones encountered in this study (nine animals). Also plotted in Fig. 2 are the mean just-discriminable differences in period length (ΔP) for four goldfish tested in similar stimulus conditions in a previous study¹¹. The behavioural ΔP values generally fall within the range of the smallest period histogram standard deviation values obtained at each frequency.

A simple temporal coding hypothesis holds that stimulus period lengths are estimated by the measurement of the temporal intervals between nerve impulses¹⁸. The discrimination problem may be viewed as a decision as to whether two samples of neural interspike intervals are drawn from the same, or two different underlying distributions. Assuming that the means of these hypothetical distributions are equal to the periods of the two signals to be discriminated, and that the distributions' variance is completely determined by the phase-locking variance, then we would expect threshold-like behaviour to occur when the difference between the means (ΔP) is approximately equal to the distributions' standard deviation (sd), that is, when $d' = \Delta P/sd = 1$ (ref. 19). The results show that this is indeed the case for the smallest sd values at each of the frequencies studied. Thus, peripheral phase-locking variance accounts well for the errors made in behavioural frequency discrimination, with the assumption that the decisions are based on the small percentage of neurones transmitting the best information.

A similar analysis was carried out by Mountcastle *et al.*¹⁸ for cutaneous flutter vibration discrimination. Human vibratory frequency discrimination performance was compared with the standard deviations of period histograms for somatosensory cortical neurones in the monkey. For vibratory frequencies between 5 and 200 Hz, the behavioural estimates of the *sds* of the underlying sensory distributions fell within the range of the smallest neural *sds*. These results for the cutaneous system are also similar to the present results in that both neural and behavioural measures of error decline approximately linearly with frequency (with unity slope in log-log coordinates), and in that the actual values of the cutaneous *sds* are only about twice as large as those of the goldfish auditory system.

Although the present results show that a simple temporal coding hypothesis accounts quite well for the behavioural capacities for frequency discrimination in the goldfish, possible alternative hypotheses are not ruled out. Further experiments are required in which phase-locking variance may be manipulated; for example, through the introduction of temporal 'jitter' in the stimulus waveform, or through the use of aperiodic stimuli such as amplitude-modulated noise²⁰. The nature of central neural mechanisms which decode the temporal input patterns remains to be explored. The goldfish seems to be a valuable preparation for combined behavioural and neurophysiological approaches to the coding of temporal patterns by nervous systems.

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Sinus node extracellular potassium transients following vagal stimulation

SEVERAL investigations have suggested a role for K^+ in the generation of the transmembrane potential during spontaneous rhythm^{1,2} and following vagal inhibition^{3,4} of the sinoatrial node (SA node) of the heart⁵. Voltage-clamp studies^{1,2} in the rabbit SA node have shown a voltage- and time-dependent increase in the outward current on depolarisation and a slow decay of the outward current on repolarisation to the holding potential. The hyperpolarisation and slowing of automaticity following vagal stimulation or acetylcholine (ACh) application is believed to be due to an increase in potassium permeability^{3,4}. The purpose of the present study was to use K^+ -sensitive microelectrodes^{6,7} to examine the time course and the magnitude of K^+ efflux and

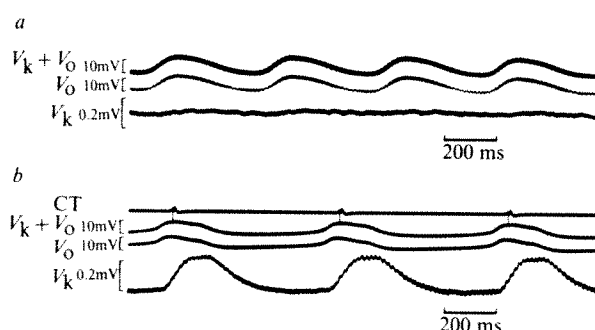


Fig. 1 *a*, The response of both barrels of a double-barrelled K^+ -sensitive microelectrode to skewed sine-wave calibrating pulses of similar time course and magnitude to extracellularly measured action potentials. Selective barrel, $V_K + V_O$; reference barrel, V_O . V_K represents the differential output showing common mode rejection at this level of resolution. *b*, The output of both barrels with the double-barrelled K^+ -sensitive microelectrode in the extracellular space of the SA node. Selective barrel, $V_K + V_O$; reference barrel, V_O . The differential trace, V_K , now shows periodic fluctuations with the same periodicity as the crista terminalis electrogram (CT) and extracellular potential fluctuations (V_O). V_K represents beat-to-beat alterations in $[K^+]_o$ and is uncontaminated by common mode rejection artefacts (see *a* above). In the V_K record 0.2 mV is equivalent to 0.034 mM potassium.

extracellular accumulation in the isolated rabbit SA node during spontaneous rhythm and following vagal stimulation. Our results confirm that K^+ permeability of the sinoatrial pacemaker cell is increased following vagal stimulation and demonstrate K^+ accumulation in the extracellular space for several seconds. Beat-to-beat extracellular potassium transients are linked to the repolarisation process as well as to diastolic depolarisation.

Experiments were carried out on rabbits (1-3 kg) anaesthetised with 10 mg per kg intravenous sodium pentobarbital. The hearts were removed and placed in Tyrode's solution, gassed with 95% oxygen and 5% CO_2 , and maintained at 37 °C; 1.0 mg l^{-1} propranolol was added to the Tyrode's to eliminate β -adrenergic influences. The atrium was opened according to the dissection of Paes de Carvalho⁸. Vagal stimulation was accomplished by a previously described⁹ modification of the technique of Vincenzi and West¹⁰. Bipolar silver electrodes were placed on the endothelial surface of the superior vena cava in the region traversed by vagal fibres innervating the sinus node area¹¹. The vagal stimuli were delivered as a train of 4-ms square-wave pulses at 100 Hz for 100-300 ms. Silver bipolar electrodes were used to record from the surface of the crista terminalis (CT) and right atrial muscle. Standard 3 M KCl-filled microelectrodes were used to record transmembrane potentials from sinus node fibres.

Double-barrelled K^+ -sensitive microelectrodes were prepared by filling the tip of one barrel of a double-barrelled glass micropipette, pretreated with a siliconising agent, with K^+ -selective resin (Corning liquid resin no. 477317 with $K^+ : Na^+$ selectivity of approximately 60:1) (refs 6, 12). The selective barrel was then backfilled to the top of the resin column with 100 mM KCl. The other barrel was backfilled with normal Tyrode's solution. K^+ electrodes with tips of 3-4 μm had a resistance of about 1,000 M Ω and an electrical response time of 15-20 ms. The chemical response time of the electrode to variation of K^+ was found to be faster¹². Selectivity values were measured before and after experiments with a series of calibrating solutions with potassium concentrations (mM) of 2.7, 10, 20, 50 and 100. Signals from the K^+ -selective barrel and the reference barrel were independently amplified by high-input impedance amplifiers ($1.5 \times 10^{12} \Omega$) with capacitance compensation. The amplified signals were differentially subtracted by a third amplifier (Bloom). Thus, we were able to obtain the potassium potential (V_K) free of the extracellular potential (V_O) fluctuations which were common to both barrels (Fig. 1*a*).

Records were simultaneously displayed on a Tektronix 5103 oscilloscope and stored on an eight-channel Hewlett Packard instrumentation tape recorder 3968A.

During advancement of the K^+ electrode into the extracellular space of the SA node, occasional large transient positive deflections on the K^+ barrel were seen. These were probably the result of the electrode rupturing cell membranes and exposing the electrode tip to the high intracellular potassium concentration. Action potentials were occasionally recorded with the double-barrelled electrode. The K^+ electrode was manipulated and either pulled back or advanced through the cell until a location was found where potential fluctuations common to both barrels were less than 10 mV. Time was allowed for elevated extracellular potassium values associated with membrane rupture to stabilise to values which corresponded to the approximate value of K^+ in the perfusate (2.7 mM).

At many of these K^+ electrode positions, beat-to-beat fluctuations in extracellular potassium were recorded (Fig. 1b). These fluctuations had the same periodicity as the spontaneous rhythm as determined by the intracellular SAN recordings and the CT electrogram (Fig. 2). The extracellular potassium concentration ($[K^+]_o$) increased gradually during phase 0 depolarisation, reaching a peak change in concentration of approximately 0.05 mM which corresponded to the middle of phase 3 repolarisation. It then subsided rapidly during the last half of phase 3 repolarisation, and the beginning of phase 4 diastolic depolarisation. There was a slower decay of potassium concentration throughout phase 4 diastolic depolarisation. The maximum peak change in K^+ concentration fluctuation in these extracellular measurements was 0.07 mM.

While continuing to monitor beat-to-beat extracellular K^+ fluctuations, vagal stimulation was applied. This was accompanied by characteristic changes in the intracellular potential response of the SA node. Initially, there was a hyperpolarisation of the maximum resting membrane potential, a decrease in the slope of diastolic depolarisation and a shortening of the action potential duration. Simultaneous $[K^+]_o$ measurements following vagal stimulation showed elevations of extracellular K^+ in the SA node of up to 0.2 mM. Following the peak accumulation, $[K^+]_o$ returned to baseline over a period of 8–13 s (Fig. 3). In some cases, $[K^+]_o$ values fell below previous baseline activity before returning to its original resting level. This potassium undershoot has previously been described by Kunze after a

period of rapid pacing in atrial muscle¹³ and by Kriz *et al.* in cat spinal cord after peripheral tetanic stimulation¹⁴. Both authors suggest that this subnormal phase of $[K^+]_o$ reflects active processes redistributing K^+ from the extracellular space.

Because of the large amount of connective tissue in the SA node^{1,2,15}, beat-to-beat fluctuations were not seen for all electrode locations⁷. In addition, in some locations where a vagus-induced $[K^+]_o$ increase is seen, beat-to-beat fluctuations are either small or below the resolution of our electrode. Furthermore, it is known that cells in the mammalian SA node are tightly packed⁵; at the point of apposition of two cellular membranes, the extracellular space takes the form of 100–150-Å-wide intercellular clefts¹⁶. The tip of the double-barrelled K^+ electrode is approximately 3–4 μ m outside diameter. Therefore, the K^+ electrode tip must rest in an artificially distorted dead space¹⁷. Thus, the efflux into the extracellular space where the electrode tip rests is substantially diluted compared with the normal cleft space. For a prolonged change in the extracellular K^+ activity of the tissue, the dead space would equilibrate with the normal extracellular concentration. However, for short-term fluctuations of K^+ activity in the intercellular cleft, the response measured by the K^+ electrode would be considerably smaller than the actual intercellular changes.



Fig. 3 Accumulation of potassium in the extracellular space of the sinoatrial node after vagal stimulation measured by a double-barrelled K^+ -sensitive microelectrode. CT, bipolar surface electrogram of the crista terminalis. SAN, transmembrane potential recording from sinus node fibre. Following vagal stimulation (VS), note the hyperpolarisation of the maximum resting membrane potential, decrease in the slope of diastolic depolarisation and shortening of the action potential duration. These events are coincident with the accumulation of K^+ in the extracellular space (V_K). Zero transmembrane potential is indicated by the upper edge of the bracket of the top calibration. 2.7 mM $[K^+]_o$ is indicated by the lower edge of the bracket of the bottom calibration.

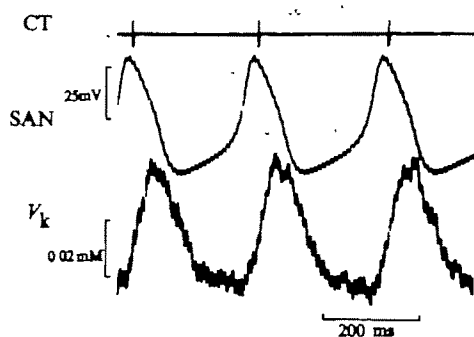


Fig. 2 Beat-to-beat extracellular potassium concentration fluctuations in the rabbit SA node during spontaneous activity measured by a double-barrelled K^+ -sensitive microelectrode. Bipolar surface electrogram of the crista terminalis at an area shown to be activated earliest during spontaneous sino-atrial conduction (CT). Transmembrane potential recording from sinus node fibre (SAN). Simultaneous potassium response was measured by subtraction of the reference barrel from the K^+ -selective barrel (V_K). As the reference barrel has been used to compensate for the electrical artefacts in the extracellular space, V_K represents the response of the K^+ -selective barrel only to changes in the K^+ concentration. The K^+ electrode is nearly linear in its response to $[K^+]_o$ for small accumulations seen during single action potentials. Zero transmembrane potential is indicated by the upper edge of the bracket of the top calibration. 2.7 mM $[K^+]_o$ is indicated by the lower edge of the bracket of the bottom calibration.

In the normal physiological range of extracellular potassium concentration, potassium-sensitive electrodes have been reported to show sensitivity to Ach fluctuations. (1 mM increase in K^+ is equivalent to the addition of 5×10^{-7} M Ach; see ref. 18.) To determine whether vagally induced release of Ach was affecting the K^+ electrode response, we added atropine to our experimental perfusate (0.2 to 1 mg l⁻¹). Atropine blocks the post-synaptic effect of Ach but not its release. We saw no vagally induced K^+ electrode response in the presence of atropine at concentrations sufficient to eliminate vagally induced rate responses. However, beat-to-beat K^+ changes were still observed.

Thus, we have confirmed the belief that the potassium permeability of the SA node pacemaker cell is increased following vagal stimulation. Extracellular potassium accumulation associated with vagal stimulation persists for several seconds and one must consider the possibility that this may contribute to membrane potential and rhythm changes after this intervention. Our direct measurement of beat-to-beat extracellular K^+ changes support the previously described observations made from voltage-clamp experiments. Beat-to-beat $[K^+]_o$ transients seem to be linked to the repolarisation process as well as to the automatic phase 4 diastolic depolarisation of the sino-auricular node of the isolated rabbit heart. This technique may thus be useful in studying the behaviour and reaction of the K^+ currents of the SA node in various conditions.

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Immunochemical demonstration of reversible reduction in choline acetyltransferase concentration in rat hypoglossal nucleus after hypoglossal nerve transection

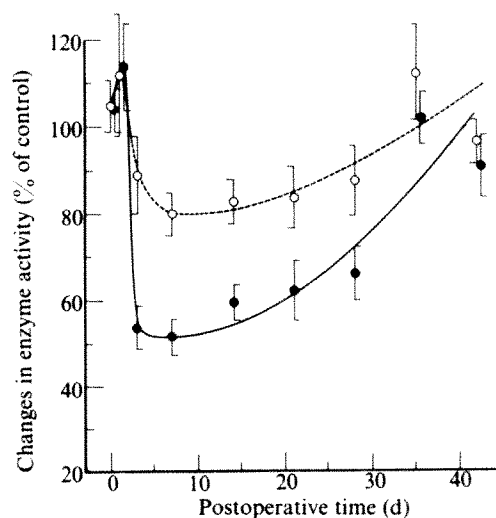
STUDIES of the cellular reaction of peripheral and central catecholaminergic neurones to axonal injury (the retrograde reaction and axon response) have demonstrated that such lesions elicit a prolonged reduction within the parent cell body in the activities and amounts of the catecholamine biosynthetic enzymes tyrosine hydroxylase (TH) and, in noradrenergic neurones, dopamine- β -hydroxylase (DBH)¹⁻³. The reduction in enzyme protein, usually reversible, lasts for several weeks and is apparently due to a decrease in the rate of enzyme biosynthesis⁴. It has therefore been proposed that a reduction in amount and activity of neurotransmitter biosynthetic enzymes may be a biochemical indicator of the retrograde reaction⁵. However, it remains to be established if a reversible diminution of neurotransmitter biosynthetic enzyme occurs during the retrograde reaction in neurones which synthesise neurotransmitters other than catecholamines. This is suggested from studies of the retrograde reaction in hypoglossal neurones, cholinergic motor neurones which innervate the tongue through the hypoglossal nerve. During the retrograde reaction elicited by lesions of the hypoglossal nerve, the activities of choline acetyltransferase (CAT), the enzyme synthesising acetylcholine, and acetylcholinesterase (AChE), the enzyme degrading it, are markedly reduced⁵⁻⁷. If the proximal stump of the lesioned nerve is allowed to regenerate and reestablish synaptic contact, AChE activity within the hypoglossal nucleus rapidly reappears⁷. It is, however, unknown whether the reduction in CAT activity is reversible and, because of a lack of specific, inhibiting antibody to CAT, whether the reduction is due to a decrease of enzyme molecules. We report here that the reduction in CAT in hypoglossal neurones elicited by transection of the hypoglossal nerve

is reversible and also, by use of a specific antibody, that it is due to a decrease in the amount of CAT enzyme protein.

All studies were carried out on adult, male Sprague-Dawley rats weighing 175-225 g. While the animals were anaesthetised with 3% fluothane in 100% O₂ blown over the nose, the hypoglossal nerve was isolated unilaterally, distal to the cricoid cartilage and the major bifurcation of the nerve. A 2-3-mm segment was excised, with special care taken to include both branches. At various days after surgery the animals were killed by cervical dislocation, and the brain was removed and dissected on cracked ice. The right and left hypoglossal nuclei were removed as a block which included parts of the dorsal motor nucleus of the vagus and the nucleus tractus solitarius, and homogenised in 50 mM potassium phosphate buffer, pH 6.8, containing 1 mM sodium EDTA, 200 mM sodium chloride and 0.4% v/v Triton X-100. CAT was assayed by the radiochemical method of Schrier and Shuster⁸, and AChE was assayed by the method of Wilson *et al.*⁹. The identity of the cholinesterase activity as true acetylcholinesterase was confirmed by the observation of 90% inhibition of cholinesterase activity by 2×10^{-7} M BW 284 C51, a specific inhibitor of true acetylcholinesterase.

Antibodies to CAT were prepared by methods described in detail elsewhere^{10,11}. In summary, the enzyme was isolated from rat caudate nuclei and first partially purified by Sepharose 4B and DEAE-cellulose column chromatography, then subjected to polyacrylamide gel electrophoresis. The single active protein band was cut from the gel, suspended in 0.9% NaCl solution, thoroughly mixed with complete Freund's adjuvant, and injected subcutaneously into rabbits every 2 weeks for a total of four treatments. The serum was collected one week after the last injection. The antibody to CAT was adjudged specific by immunoelectrophoresis against crude and partially purified CAT from rat caudate nucleus and that the antibody inhibited only the activity of CAT in purified form or in brain homogenates.

Fig. 1 Time course of changes in activities of CAT (closed circles) and AChE (open circles) in the ipsilateral hypoglossal nucleus following unilateral lesion of the hypoglossal nerve. Enzyme activity is expressed as % of mean activity in the contralateral control hypoglossal nucleus. Neither the activities of AChE nor CAT changed in the control hypoglossal nucleus throughout the time course studied. Each point represents the mean $\pm$ s.e.m. of 6-8 animals. The specific activities of CAT and AChE in the left and right hypoglossal nuclei of control (unoperated) rats were as follows: CAT (left/right), $80 \pm 7/74 \pm 7$ nM per mg protein per 10 min; AChE (left/right) $1.9 \pm 0.1/1.9 \pm 0.1$ μ M per mg protein per 10 min.



Immunotitration was carried out by adding increasing amounts of antiserum to CAT to 80- μ l aliquots of appropriately diluted supernatants of rat hypoglossal nucleus homogenates. Aliquots of 1:1 preimmune serum diluted with distilled water were added to each sample to bring the final volume to 90 μ l. The mixture was then agitated and allowed to stand for 1 h at room temperature with occasional shaking, incubated overnight at 4 °C, and centrifuged at 9,150g for 10 min. A 75- μ l aliquot of the supernatant was removed for assay of residual CAT activity.

Other rats subjected to hypoglossal nerve lesion were perfused with 10% formalin, the brains were removed, further fixed in Bouin's solution and embedded in paraffin. Ten- μ m sections were made through the hypoglossal nucleus, stained with cresyl violet and then examined by light microscopy.

Figure 1 shows changes in the activity of CAT in the hypoglossal nucleus at different days after hypoglossal nerve section. No change was observed after 24 h, but by 3 d the activity had fallen to 50% of control. Five weeks after the lesion, activity had returned to control levels. CAT activity did not change in the contralateral hypoglossal nucleus. Hypoglossal nerve section also produced reversible changes in AChE activity, similar in time course to but smaller than that of CAT (Fig. 1), confirming, in part, the findings of others^{6,7}.

To determine whether the transient reduction in CAT activity in the hypoglossal nucleus after hypoglossal nerve section was due to a decrease in specific enzyme protein or to an inactivation of pre-existent enzyme molecules, the degree of inhibition by antibody to CAT activity in homogenates of control hypoglossal nuclei was compared with that of nuclei whose hypoglossal nerve had been sectioned 7 d previously. The results of a typical experiment are shown in Fig. 2. As increasing amounts of antiserum were added to a fixed quantity of homogenate, CAT activity in the supernatant decreased. The slopes and equivalence points (that is, that quantity of antibody which just saturates the available antigen) of immunotitration curves for both experimental and control hypoglossal nuclei were quite similar. These results indicate that the reduction in CAT activity in the hypoglossal nucleus following hypoglossal nerve section is due to a reduction in the concentration of enzyme protein.

During the retrograde reaction, minimal changes of chromatolysis were observed in the affected hypoglossal nucleus. These changes consisted of a slight increase in nucleolar size and darkening of chromatin. No loss of neurones was noted based on careful counts of neurones in randomly selected sections through the nucleus.

The present study shows that the reduction in CAT activity in the hypoglossal nucleus during the retrograde reaction is entirely reversible. The immunotitration studies demonstrate that the reduction in perikaryonal CAT activity following hypoglossal nerve lesion is a result of a diminution in the quantity of CAT protein rather than inactivation of the enzyme. This reversible reduction in enzyme protein may be due to either a reduction in CAT synthesis or an increase in the rate of degradation of the enzyme. The observation that the reversible reduction in DBH which occurs in central noradrenergic neurones is entirely due to a diminished rate of synthesis⁴ suggests a reduction of synthesis of this specific protein. It is intriguing that the time courses of the reversible reductions in AChE and CAT activities in the hypoglossal nucleus following axotomy are so similar. Many studies suggest that AChE is transported along the axon at a very rapid rate, whereas CAT is transported at a predominantly slow rate⁵. Despite these apparent differences in axonal transport rates, our data suggest that the two enzymes may have similar control mechanisms in the cell body.

Thus, like noradrenergic neurones arising in the locus coeruleus³, and central dopaminergic neurones⁸, injury to axons of the hypoglossal nerve, a cholinergic motor nerve, results in a reversible reduction in the capacity of the neurone to accumulate the enzyme which catalyses the biosynthesis of the neurotransmitter as well as the one degrading it. However, others have shown that in the hypoglossal nucleus during the period of

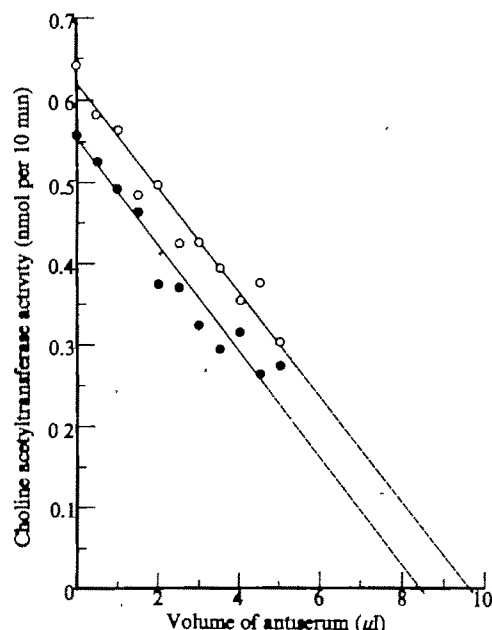


Fig. 2 Immunochemical titration of choline acetyltransferase in the hypoglossal nucleus from control (open circles) and experimental (closed circles) rats. The hypoglossal nerve ipsilateral to the experimental hypoglossal nucleus had been sectioned 7 d earlier. The homogenate of the control nucleus was diluted 1:2 so that the enzyme activity per unit volume was approximately equal in the experimental and control preparations. Note that the slopes and equivalence points are nearly identical. Linear regression analysis of each curve produced the following equations: control, $y = -0.065x + 0.621$; experimental, $y = -0.066x + 0.553$.

reduction in AChE and CAT activities there is an increase in the rate of incorporation of labelled amino acid into protein¹³ as well as an increase in the total protein content per cell¹⁴. Thus, the reduction in neurotransmitter-metabolising enzymes in cholinergic cell bodies is probably highly selective, involving a very small percentage of the total amount of protein synthesised in the cell body. These results and those of previous studies on central catecholaminergic neurones suggest that a reversible reduction in transmitter-synthesising capacity in the cell body may be a sensitive and specific indicator of axonal damage. It seems likely that during the 2–4-week period following axotomy the first priority of the neurone is to regenerate its axons and in the case of peripheral neurones like the hypoglossal, to re-establish synaptic contacts. During this recovery phase, protein synthesis associated with regeneration seems to establish priority over protein synthesis subserving neurotransmission.

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Membrane specialisations between demyelinated axons and astroglia in chronic EAE lesions and multiple sclerosis plaques

AXOLEMMAL specialisations are known to exist along myelinated fibres in the adult vertebrate central nervous system (CNS), particularly at nodes, between the axon and the oligodendrocyte, and at presynaptic sites¹. However, modified regions of axonal membrane have not been described in relation to astroglial cells. In demyelinated lesions in the CNS, it has always been assumed that the naked segment of an axon, although intimately invested by glial scar tissue, shares no junctional relationships with its scarring environment. This report documents for the first time membrane specialisations between naked CNS axons and fibrous astroglial cell processes, developing subsequent to demyelination. These structures may have physiological implications.

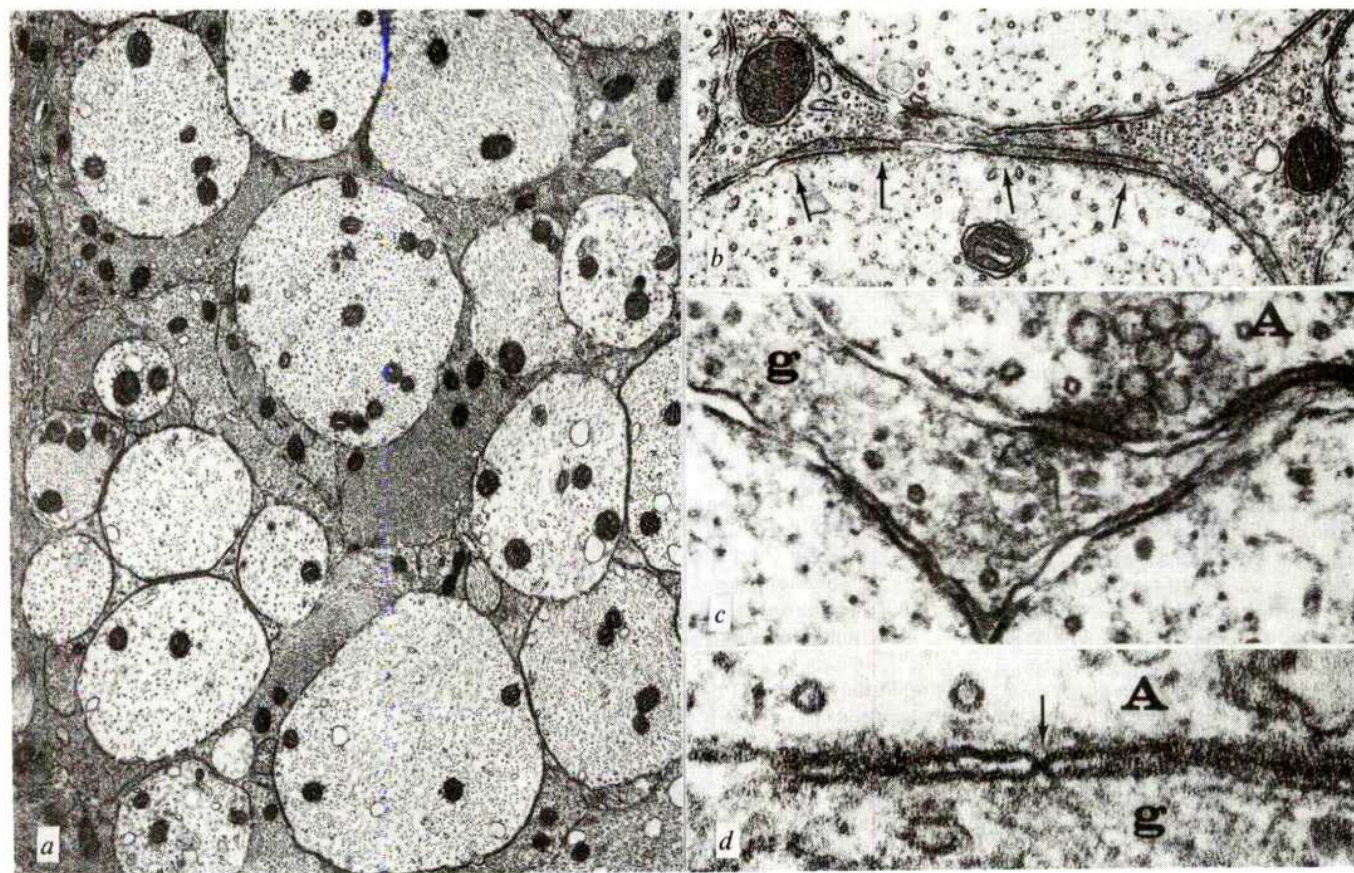
Spinal cord tissue from two demyelinating conditions have been examined. First, from strain 13 guinea pigs affected with chronic relapsing experimental allergic encephalomyelitis (EAE)², and second, from a case of chronic multiple sclerosis (MS), the paradigm of the human CNS demyelinating diseases³.

Chronic relapsing EAE was induced by sensitisation of juvenile strain 13 guinea pigs with isogeneic spinal cord tissue in complete Freund's adjuvant². Typically, animals developed delayed onset neurological signs (incontinence, paraparesis, and

so on) some 8–12 weeks post-inoculation (PI). These signs remitted, albeit incompletely, and were usually followed by several relapses. This clinical course is highly reminiscent of that of MS, for which the disease serves as a major experimental analogue. In the present study, five animals have been studied. One was sampled at the first onset of signs (10 weeks PI), three after a single relapse (12–24 weeks PI), and one after two relapses (20 weeks PI). Slices of spinal cord from L₆, L₇ and S₁ were obtained and processed for ultrastructural study after whole body perfusion with 5% glutaraldehyde buffered with PO₄, osmication, dehydration and embedding in Epon⁴. Human CNS tissue was obtained at autopsy from a patient who had suffered from chronic relapsing MS for more than 20 yr, the autopsy being carried out some 30 min post-mortem. In the lumbar cord at L₃, a large demyelinated plaque was grossly visible in the anterior column. Thin transverse slices across this lesion were taken and immersed in glutaraldehyde for 60 min and then processed as above for electron microscopy.

Demyelinated plaques of different ages were common in spinal cord tissue of the guinea pig. They were usually subpial and displayed varying degrees of developing disease according to the frequency of relapses and recurrent inflammatory activity. Lesions were occasionally grossly visible. Chronically demyelinated plaques consisted of longitudinally arranged naked axons invested by fibrous astroglial tissue (Fig. 1a). Axons were readily distinguishable from astroglial cell processes on the basis of cross-bridged neurofilaments, abundant microtubules and a circular profile^{1,5}. Astroglial processes were flattened, contained a preponderance of tightly packed filaments (not cross-bridged), fewer microtubules, and had a tendency to invest axons in an

Fig. 1 a, Low power electron micrograph showing a group of transversely sectioned chronically demyelinated axons from a subpial lesion in the lateral column of a spinal cord at L₇. Note how the axons are intimately embedded in a matrix of fibrous astroglial processes. Chronic EAE, 16 weeks PI, one relapse. $\times 12,000$. b, Higher magnification of a demyelinated axon from a dorsal column lesion at L₇ shows regional enhancement of the axolemma due to thickening of the membrane and an associated subaxolemmal coating of granular material. In two places (arrows) corresponding thickening of the parallel arranged membrane of an investing astroglial cell process is shown, between which a cleft containing electron-dense material is present. Chronic EAE, 20 weeks PI, two relapses. $\times 35,000$. c, An astroglial process (g) separates three naked axons, between one of which (A) a synapse-like junction is seen. Note the vesicles in the axoplasm of the affected axon (A). Same preparation as Fig. 2. $\times 100,000$. d, The axolemma of a demyelinated axon (A) above appears to form a fenestra (arrow) with the membrane of an astroglial cell process (g). Same preparation as Fig. 2. $\times 200,000$.



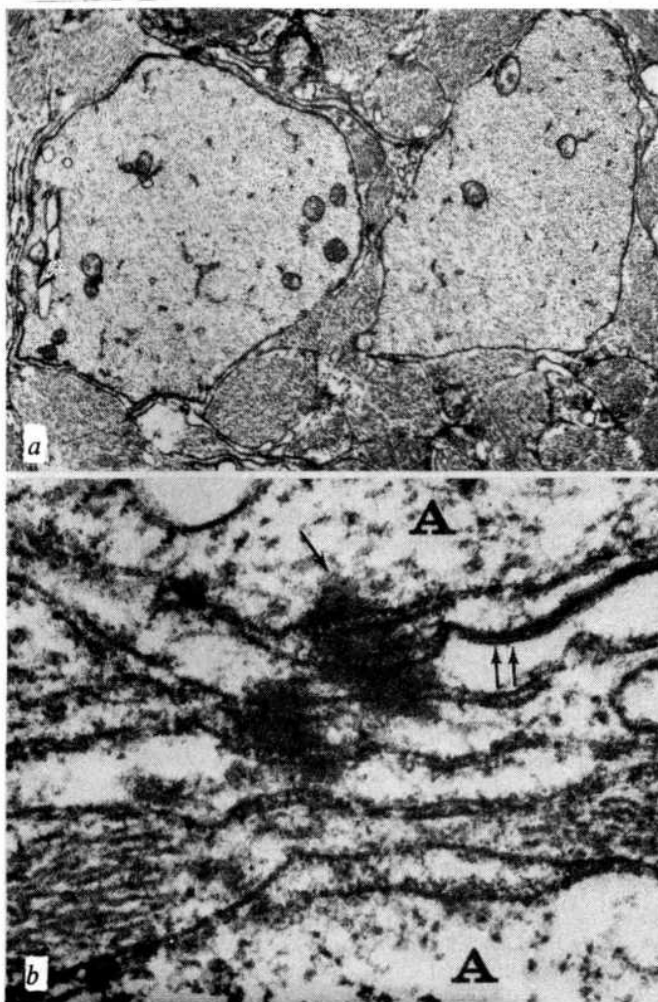


Fig. 2 *a*, Low power view of a chronic MS plaque in an anterior column of the spinal cord at L₃. Note the intense glial scarring and the two demyelinated axons in transverse section. Chronic MS, over 20 yr duration, multiple relapses. $\times 12,000$. *b*, Detail from the above chronic MS plaque showing two axons (A) above and below, one of which displays a small desmosome-like junction which contacts others traversing several interposing fibrous astroglial cell processes. A gap junction is also seen (double arrows). $\times 60,000$.

haphazard fashion. Closer examination of the naked axons revealed regional densifications of the axon membrane on the axoplasmic side of which a narrow zone of osmiophilic granular enhancement was associated (Fig. 1*b*). Not infrequently, these specialised regions of axolemma apposed a similar region of densification on the investing astroglial membrane, and between the two, a 20–40 nm cleft within which a floccular substance could be discerned. These axon–astroglial membrane specialisations were very common (occurring in more than 60% of affected axons) and were present in all chronically demyelinated EAE lesions. They were not seen in normally myelinated regions, remyelinated zones, or in areas of recent demyelination. On two occasions, axons were seen to possess primitive ‘synapse-like’ junctions with investing astrocytic processes (Fig. 1*c*). These consisted of small desmosome-like structures in association with which were 60–80 nm diameter vesicles in the juxtaposing axoplasm. In addition, three axons were seen to possess fenestrae between adjacent astroglial cell processes. These occurred along zones of specialised axolemma where the membranes of the axon and the astrocyte fused to form a single unit membrane (Fig. 1*d*). Whether or not these fenestrae represented artefacts of sectioning remains to be elucidated by goniometer stage techniques (tilting). Elsewhere throughout the EAE plaques, a variety of junctional complexes was encountered between astroglial cell processes, particularly desmosomes, and gap and punctate junctions.

In the examined MS plaque, demyelinated axons were tightly packed in an intense fibrous astroglial matrix (Fig. 2*a*). Membrane imperfections due to the poorer fixation conditions were apparent. In contrast to the EAE material, extensive regions of membrane specialisation between axolemmae and adjacent astrocytes were not seen. Instead, more than 30% of the axons examined were seen to contact astroglia by means of small desmosomal junctions—regions of membrane some 150–200 nm in extent beneath which were broad zones of electron dense granular material. Sometimes, these complexes occurred in short series from an axon across several investing glial cell processes (Fig. 2*b*). In addition, many naked axons showed extensive subaxolemmal thickening, typical of that seen at nodes¹. Synapse-like arrangements were not encountered between axons and astroglia. Gap junctions (Fig. 2*b*) and small desmosomes were common between astroglial cell processes.

The present observations of membrane specialisations and possible junctional complexes between demyelinated axons and scarring glial cell processes in the adult CNS are unprecedented in neuropathology. However, somewhat similar membrane specialisations have been described previously in the spinal cord of the mouse during development, and have been termed puncta adherentia (*sic*) and axoglial synapses⁶. These connections represented a transient phenomenon and were not present after embryonic day 15. Their appearance was speculated “to reflect errors in development”, although their disappearance might have been linked to the development of more specific mechanisms. These unusual thickenings might represent transient phenomena or biological errors since they do not occur along normal or remyelinated fibres. The MS plaque under study was probably many years old (compared with the 2–6 months of the EAE plaques), and the areas of membrane specialisation may have undergone some further modification with time.

That these abnormal membrane relationships between axons and glia are the result of prolonged absence of myelin seems incontrovertible and they probably play some supportive role for the axon. However, whether they impart a compensatory property on the already impaired nerve fibre conduction remains to be established. In view of the frequent lack of correlation between clinical status and the extent of damage in the demyelinated CNS, the possibility that scarring astrocytes somehow unify naked fibres and are capable of participation in neural transmission, should be considered. Although the final characterisation of these junctional complexes must await freeze-fracture analysis⁷, on the basis of the frequency of these morphological peculiarities, retrospective examination of other models of CNS demyelination⁸ will reveal whether or not these specialisations are features only of immune-mediated demyelination.

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Supersensitivity to the cyclic GMP response to glutamate during cerebellar maturation

THE action of certain neurotransmitters on receptors is associated with changes in the levels of cyclic nucleotides. Catecholamines, for example, increase cyclic AMP levels in both the central and peripheral nervous systems¹. In the adult brain the cerebellum contains the highest levels of cyclic GMP (ref. 2), and these can be altered *in vivo* by manipulation of excitatory pathways^{3,4} and intraventricular injection of γ -aminobutyric acid or glutamate^{3,5}, and *in vitro* by certain amino acid transmitter candidates^{6,7} and depolarising agents⁸. We show here that during a relatively short period in postnatal development, the excitatory amino acid, glutamate, elicits very large increases in the levels of cyclic GMP in slices of rat cerebellum, apparently located in the cells differentiating at this time.

Cerebella were rapidly excised from rats (Porton strain) of various ages, chopped (0.4 × 0.4 mm) at room temperature using a McIlwain chopper, and incubated at 37 °C in Krebs solution. The tissue was inactivated by boiling and, after homogenisation and centrifugation, cyclic nucleotides were estimated in the supernatant using commercial kits (Radiochemical Centre).

On addition of glutamate, cyclic GMP levels increased to reach a peak after 2–5 min irrespective of age (the time course at 8 d is shown in Fig. 1a). The extent to which they increased, however, was markedly age dependent. At 8 d (birth = day 1), maximal changes occurred at a glutamate concentration of 3 mM (Fig. 1b), with levels ($\pm$ s.e.m.) increasing from 0.56 ± 0.07 to 129 ± 5 pmol per mg protein, representing a >200-fold stimulation. This is considerably larger than any yet reported for non-tumorous nervous tissue. Changes of this magnitude were confined to slices from 8–14-d-old rats (Fig. 2). Either side of

Fig. 1 a, Time course and b, concentration dependence of the increase in cyclic GMP elicited by glutamate in slices of cerebellum from 8-d-old rats. Slices were preincubated (at about 1 mg protein ml⁻¹) for about 1.5 h (to allow cyclic nucleotide levels to stabilise) in Krebs solution containing (mM): NaCl (118), KCl (4.7), CaCl₂ (2.5), NaHCO₃ (25), KH₂PO₄ (1.18), MgSO₄ (1.19) and glucose (11), continuously gassed with a mixture of 95% O₂/5% CO₂ (pH 7.4) in flasks in a shaking water bath at 37 °C. At various times (a) or 5 min (b) after addition of glutamate, slices were removed using a Pasteur pipette, separated from the suspending medium by filtration and plunged into 1 ml of 50 mM Tris, 4 mM EDTA buffer, at 100 °C for 3–5 min. The interval between removal of slices and inactivation was <5 s. After homogenisation (through narrow gauge needles) and centrifugation, cyclic nucleotides in the supernatant fluid were measured. The protein content of the homogenate was estimated using the method of Lowry¹⁸. Points are the mean of 4–20 experiments, except for the 15-min point in a (mean of 2). Error bars are s.e.m. where this exceeds the dimensions of the symbol.

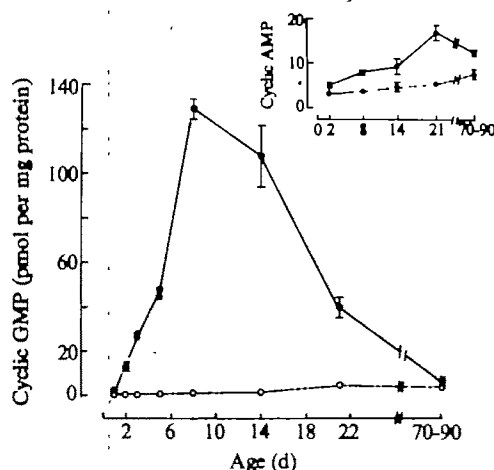
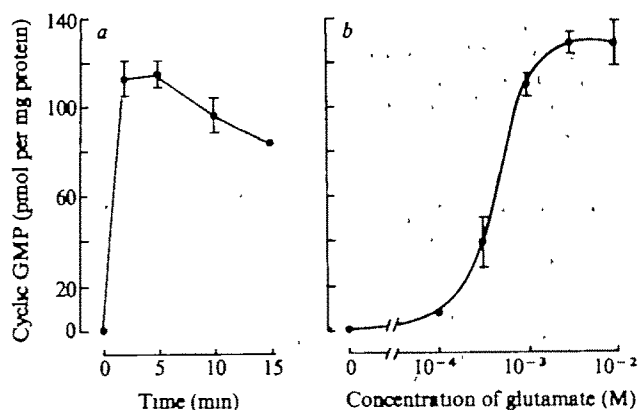


Fig. 2 Dependence on age. Solid circles show the cyclic GMP content in slices of cerebellum 5 min after the addition of glutamate (3 mM). Open circles are unstimulated levels, which show a developmental pattern similar to that found *in vivo* in mouse cerebellum², being fairly constant up to 8 d (at about 0.5 pmol per mg protein), increasing at 14 d (1.4 pmol per mg protein) and reaching adult values by 21 d (3–4 pmol per mg protein). At 1, 3 and 5 d, points show results of two experiments; at other ages, $n = 4-20$. The insert shows the corresponding levels of cyclic AMP (pmol per mg protein) with respect to age. Error bars are s.e.m. where this exceeds the dimensions of the symbols.

this period, the degree of stimulation fell markedly, such that in the newborn it was sixfold, at 21 d tenfold, and in the adult (70–90 d) only twofold. Cyclic AMP levels also increased in response to glutamate, but in contrast, these were similar for all ages studied, and small, only about twofold (Fig. 2).

An important consideration in interpreting these results is the relative degree of preservation of the cells and their processes in the incubated slices with respect to age. The observed developmental pattern of the cyclic GMP response up to 21 d is unlikely to reflect variation in tissue damage, as electron microscopy of slices incubated for 1.5–2 h showed good preservation of morphology up to 21 d, but in slices from older rats, swelling and pyknosis in neuronal and glial structures became evident, suggesting that the response observed with adult slices may be an underestimate.

The question arises as to the possible localisation of the response. In the rat cerebellum, 97% of the cells are formed postnatally⁹. At 8 d, cell proliferation, including that of the precursors of the granule cells, the most abundant cell type in the adult cerebellum, is extensive. The following experiments were carried out in an attempt to narrow down cell types possibly responsible for the increase in cyclic GMP.

The dividing cells were eliminated *in vivo* using the mitotic inhibitor hydroxyurea (HU); injection of HU at 6 d (2 mg per g body weight) results in an almost complete loss of the external germinal layer by 8 d^{10,11}, corresponding to a 35% decrease in cell numbers compared with controls (in terms of DNA content¹¹). In slices from 8-d-old, HU-treated rats, however, both basal and maximal glutamate-stimulated cyclic GMP levels were increased by 100% and 80%, respectively, compared with slices from untreated rats (Table 1a), indicating a localisation predominantly in those cells undergoing differentiation as opposed to proliferation.

The intracerebellar nuclei were not a major site of the response, as the increase of cyclic GMP levels in slices from the ventral two-thirds of the cerebellum (where the nuclei are found) was similar to that in slices from the dorsal one-third (in which the nuclei would be excluded) and from whole cerebellum (Table 1b).

In addition, if the response resided in the mature granule cells (excitatory interneurons), it would be anticipated (unless the early-maturing granule cells are unusual) that the measured

Table 1 Effect of removal of proliferating cells and intracerebellar nuclei on basal and glutamate-stimulated levels of cyclic GMP

	<i>n</i>	Cyclic GMP (pmol per mg protein)	
		Basal	+ Glutamate (3 mM)
<i>a</i> Treatment			
Saline	4	1.41 ± 0.13	167 ± 8
Hydroxyurea	4	2.87 ± 0.52	306 ± 13
<i>b</i> Part of cerebellum			
Whole	2	1.24, 0.84	131, 136
Dorsal, one-third	2	1.35, 1.32	141, 142
Ventral, two-thirds	2	0.92, 0.66	115, 132

In *a*, rats were injected on day 6 either with hydroxyurea (2 mg per g body weight, intraperitoneally) to delete proliferating cells, or with an equivalent volume of saline; cerebella were used 2 d later. In *b*, either whole cerebella (8 d) were chopped, and incubated as usual, or the dorsal one-third was separated by hand using a razor blade, and chopped and incubated separately from the ventral two-thirds. (The intracerebellar nuclei are found in the latter part.) Data in *a* are given as mean ± s.e.m.; in *b* individual results are shown.

degree of stimulation would parallel the large increase in their numbers over 7–15 d after birth¹², contrary to our findings.

Consequently, much of the glutamate-induced increase in cyclic GMP in the immature cerebellum is likely to originate in the cells formed prenatally and/or in cells formed before the eighth day (Purkinje cells, Golgi cells, inhibitory interneurons, glial cells).

The period of maximal elevation of cyclic GMP levels by glutamate (8–14 d) coincides with extensive morphological, electrical and biochemical maturation of the cerebellum¹³; by 21 d, the tissue is essentially mature. Assuming the response to be localised in the neurones, the magnitude of the observed changes in cyclic GMP and their confinement to this period in development might have implications regarding a role for transmitter-induced changes in cyclic GMP in maturing neurones in the cerebellum, for example, in the stabilisation of synapses¹⁴. We would add that this apparent 'supersensitivity' to glutamate does not extend to other putative neurotransmitters with action supposedly linked to cyclic nucleotide-generating systems. Changes in cyclic AMP levels induced by noradrenaline at 8 d (maximally a two- to threefold increase, J.G. and R.B., in preparation) were similar to those reported for the adult^{15,16} and, in contrast to the action of acetylcholine in slices of adult rabbit cerebellum¹⁷, the cholinergic agonist, carbachol, at concentrations of up to 1 mM, produced no significant change in cyclic GMP levels in slices of rat cerebellum at 8 d (J.G. and R.B., in preparation).

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Calcium-dependent protein phosphorylation during secretion by exocytosis in the mast cell

IN many secretory cells materials destined for secretion are packaged in membrane-limited granules and are extruded by exocytosis¹. This process is known to require metabolic energy and calcium^{2–6}. Calcium probably acts as a common mediator in stimulus-secretion coupling², but the molecular mechanisms involved are unknown. Increasing evidence that many physiological processes are regulated by phosphorylation of proteins⁷ has led to the suggestion that calcium-dependent phosphorylation of proteins may be involved in secretory responses^{8–13}. We have sought evidence for this possibility in the mast cell, the rapid and extensive secretory activity of which conforms to the general pattern of exocytosis dependent on energy and calcium^{4,14–20}. Here we report a rapid phos-

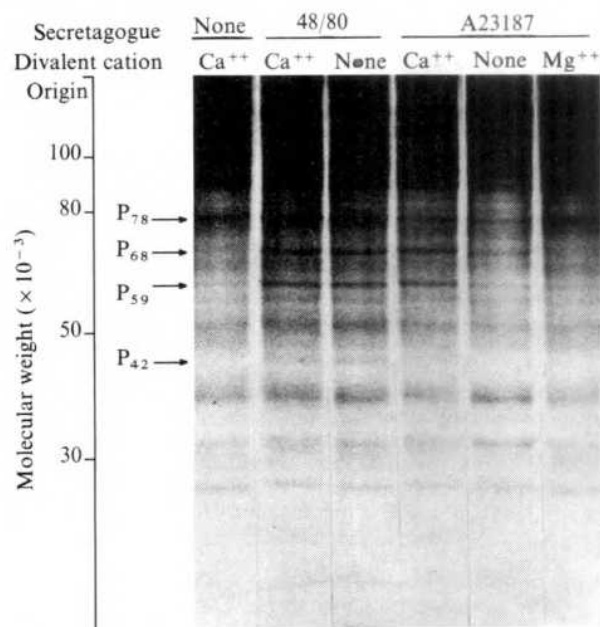
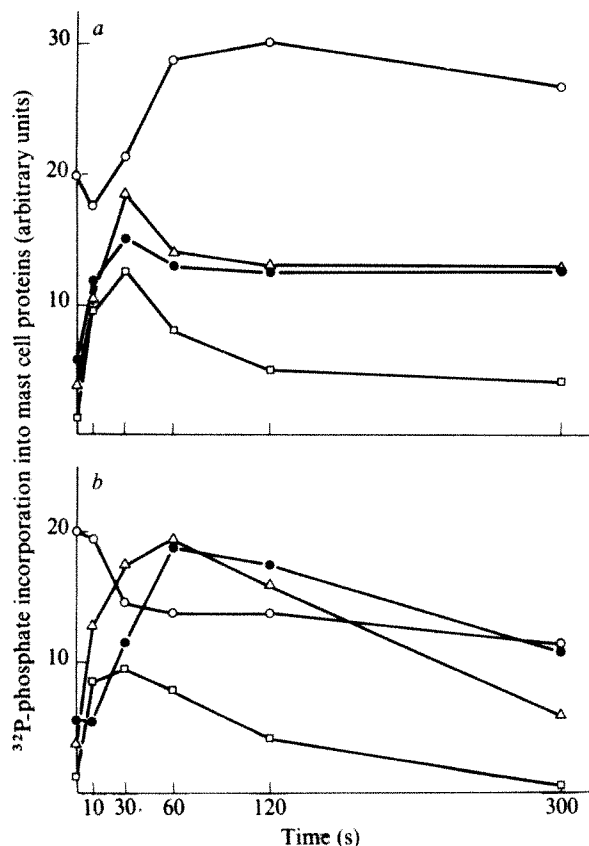


Fig. 1 Effect of 48/80 and A23187 on endogenous phosphorylation of mast cell proteins. Peritoneal mast cells derived from 15 Sprague-Dawley male rats (~400 g) were collected and purified as described previously²⁸. Mast cells (80–90% purity, 1.5×10^6 cells ml⁻¹) were preincubated in a standard buffer (HEPES 5 mM, pH 7.0, NaCl 150 mM, KCl 5 mM and glucose 5.5 mM) in the presence of CaCl₂ 1 mM, 0.1% bovine serum albumin and 0.75 mCi ml⁻¹ carrier-free ³²P-phosphate (NEN, 9120 Ci mmol⁻¹) for 1 h at 37°C. The cells were centrifuged at 180g for 5 min at 4°C and resuspended in 5 ml standard buffer in the presence of 0.1 mM EGTA. The cells were centrifuged twice more, each time followed by a resuspension in the standard buffer. Mast cell suspensions (0.2 ml) were introduced into plastic tubes containing 48/80 1 µg ml⁻¹, A23187 1 µg ml⁻¹, calcium 1 mM or magnesium 1 mM, in 0.1 ml standard buffer. Following 1 min incubation at 37°C, 150 µl of a stop solution (10% sodium dodecyl sulphate (SDS; w/v), Tris-HCl 100 mM, pH 7.4, β-mercaptoethanol 5 mM, sucrose 0.1 g ml⁻¹ and bromophenol blue tracking dye 0.02 mg ml⁻¹) was added and the tubes were placed in a boiling water bath for 2 min. Electrophoresis on 10% polyacrylamide gels, protein staining of the gels, and autoradiography were carried out as described previously¹³. All radioactive bands disappeared after treatment of the samples with pronase but not with ribonuclease, using conditions described previously¹³, and thus represent phosphoproteins. The apparent molecular weights of the phosphorylated protein bands indicated by the arrows (78,000, P₇₈; 68,000, P₆₈; 59,000, P₅₉; 42,000, P₄₂) were determined by calibrating the gel with standard proteins of known molecular weight. Degranulation, used as a measure of mast cell secretion, was monitored by light microscopy²².

phorylation of several proteins in rat peritoneal mast cells stimulated to secrete either by the classic mast cell secretagogue 48/80 (refs 15 and 21–23) or by the calcium ionophore A23187 (refs 22–25).

Purified rat peritoneal mast cells, which had been preincubated for 1 h with $^{32}\text{P}_i$, were incubated for 1 min with 48/80 or A23187 in the presence or absence of calcium or magnesium. The cells were then dissolved in sodium dodecyl sulphate and subjected to polyacrylamide gel electrophoresis (PAGE) and autoradiography. Addition of compound 48/80 $1\text{ }\mu\text{g ml}^{-1}$, which caused extensive degranulation of the mast cells within 1 min, resulted in an increased phosphorylation of four proteins (of apparent molecular weights 78,000, 68,000, 59,000 and 42,000) (Fig. 1, compare lanes 1 and 2). This change in phosphorylation elicited by 48/80 did not require the presence of extracellular calcium (Fig. 1, lane 3). In these experiments, mast cell secretion elicited by 48/80 also occurred in the absence of calcium, in agreement with evidence that this secretagogue acts largely through mobilisation of intracellular calcium^{22,23,25,26}. The general pattern of phosphorylation seen in response to 48/80 was highly reproducible (14 experiments), although the increase in radioactive phosphate incorporated into these four proteins varied from one experiment to another. The phosphorylation of several other proteins was sometimes influenced by 48/80 (and A23187), but this effect was not consistent.

Fig. 2 Phosphorylation of mast cell proteins as a function of incubation time with 48/80 (a) or A23187 (b). Mast cells (0.2 ml), isolated and purified as described in Fig. 1, were introduced into plastic tubes containing either 48/80 $1\text{ }\mu\text{g ml}^{-1}$ or A23187 $1\text{ }\mu\text{g ml}^{-1}$, in 0.1 ml standard buffer containing calcium 1 mM. At the times indicated, 150 μl of stop solution was added and the phosphorylation of mast cell proteins was analysed by SDS-PAGE and autoradiography¹³. The amount of ^{32}P -phosphate incorporated into four proteins, P_{78} (○), P_{68} (△), P_{59} (●) and P_{42} (□), was determined by scanning the autoradiographs with a Canaco G-II microdensitometer. The results are expressed in arbitrary units²⁹. Results qualitatively similar to those shown here were obtained in four other experiments.



Phosphorylation of the proteins with apparent molecular weights of 68,000, 59,000 and 42,000 was evident within 10 s after addition of 48/80, whereas phosphorylation of the 78,000 protein was not evident until 30–60 s after addition of the secretagogue (Fig. 2). After being maximally phosphorylated, these four proteins displayed different rates of dephosphorylation. The fact that compound 48/80 binds almost exclusively to receptors on mast cell membranes²⁷ indicates that the proteins phosphorylated in response to this secretagogue are derived from mast cells rather than from other contaminating peritoneal cells (10–20%).

As in the experiments with 48/80, mast cells incubated with A23187 $1\text{ }\mu\text{g ml}^{-1}$ in the presence of calcium 1 mM showed increased phosphorylation of the proteins with apparent molecular weights of 68,000, 59,000 and 42,000 (Fig. 1, compare lanes 1 and 4). When calcium was omitted from the incubation medium, A23187 had a diminished effect on the phosphorylation of these proteins (Fig. 1, lane 5). In the absence of calcium, mast cell secretion was also diminished, in agreement with studies demonstrating only a feeble stimulant effect of A23187 on secretion under similar conditions^{24,25}. The residual effect on mast cell secretion and on protein phosphorylation seen with A23187 in calcium-free medium was abolished by magnesium (Fig. 1, lane 6). Magnesium has been shown to reduce residual mast cell secretion in a low calcium medium²⁴. The phosphorylation of the 78,000 band was decreased by A23187 both in the presence and in the absence of calcium but not in the presence of magnesium (Fig. 1). The time course of phosphorylation and dephosphorylation of the 68,000, 59,000 and 42,000 proteins (but not that of the 78,000 protein) in response to A23187 and calcium was generally similar to that observed in the presence of 48/80 (Fig. 2).

These results demonstrate that both 48/80, which is thought to mobilise calcium from intracellular stores, and A23187, which promotes entry of calcium into the cell, stimulate the phosphorylation of the same proteins at a time when the secretory response is still developing. Calcium-dependent protein phosphorylation may therefore be involved in stimulus-secretion coupling. As yet, however, the evidence that the two phenomena are causally linked is only suggestive.

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Bromocriptine-induced inhibition of plasma dopamine, noradrenaline and adrenaline responses to LH-RF

In addition to their regulatory role on pituitary hormones, hypothalamic-hypophyseal regulatory hormones may function in brain as neurotransmitters. Thus, in addition to the considerable evidence in laboratory animals suggesting that the release of luteinising hormone releasing factor (LH-RF) is regulated by brain amines¹, further evidence suggests that LH-RF may function at central nervous system synapses. It is found within synaptosomes and vesicle-like structures in various areas of brain²; it influences animal behaviour through a direct action in brain³, and microiontophoretic applications of LH-RF to neurones in the mediobasal hypothalamus and preoptic area are associated with prominent short-term changes in neuronal excitability, the most frequently observed effect being decreased excitability⁴. It has been suggested that axon collaterals within a putative peptidergic neuronal network may function in feedback circuits regulating activity of the tuberoinfundibular system and to inform other brain regions about the activity of this system. It seems reasonable to speculate that LH-RF release from such peptidergic neurones may act as a neurotransmitter or modulator to regulate the activity of certain central aminergic neurones. Thus, we examined the effects of LH-RF on plasma catecholamines in normal men, and found a rapid decrease in plasma concentrations of dopamine, noradrenaline and adrenaline (Fig. 1). Furthermore, bromocriptine, a dopamine receptor agonist, entirely prevented this depressant effect of LH-RF on plasma catecholamines.

In men kept supine for 2 h in the absence of bromocriptine, basal plasma concentrations of dopamine, noradrenaline and adrenaline were 58 ± 1 (mean $\pm$ s.e.), 273 ± 3 and 92 ± 4 pg ml⁻¹, respectively. These are similar to values we have reported elsewhere^{6,7}. LH-RF produced rapid decreases in plasma concentrations of all three catecholamines (Fig. 1). Plasma concentrations of dopamine, noradrenaline and adrenaline 5 min after LH-RF administration were 65 ± 3 , 77 ± 3 and 48 ± 2 % of the pre-LH-RF basal levels, and 20 min after LH-RF, decreased further to 34 ± 3 , 35 ± 1 and 24 ± 0.5 % of basal levels.

We have reported elsewhere that bromocriptine decreases plasma concentrations of dopamine, noradrenaline and adrenaline⁷. However, 4 h after bromocriptine administration, plasma concentrations of all three catecholamines had returned to basal levels. LH-RF administered at this time failed to suppress plasma concentrations of dopamine, noradrenaline and adrenaline (Fig. 1). Thus, bromocriptine interfered with the effect of LH-RF on plasma catecholamines at a time when its own effect on catecholamine release had apparently ended, although its effect on serum prolactin was still present.

Bromocriptine seems to produce many of its effects through an action on dopamine receptors⁸. Although evidence has been presented for an action on postsynaptic dopamine receptors⁹, more recent evidence supports an effect on presynaptic receptors¹⁰. We have suggested that bromocriptine may decrease plasma catecholamine concentrations by an action on presynaptic receptors of unknown central catecholaminergic neurones mediating sympathetic outflow, although a similar action on peripheral neurones could not be ruled out⁷.

It seems reasonable to speculate that LH-RF may act in a manner similar to that of bromocriptine. Alternatively, LH-RF

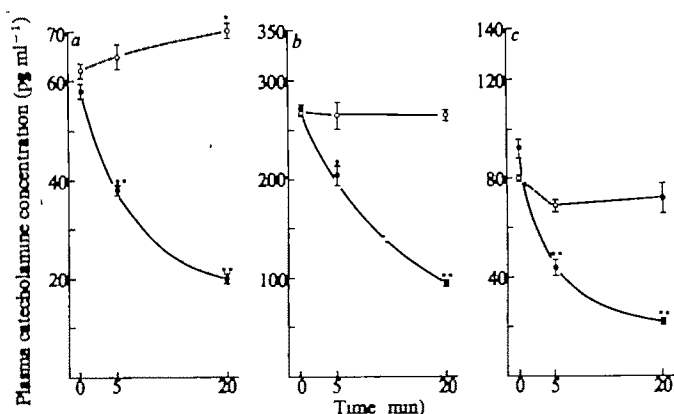


Fig. 1 Plasma dopamine, noradrenaline and adrenaline responses to LH-RF in the absence (●) and presence (○) of bromocriptine. Studies were carried out in three normal men, aged 36, 38 and 50 yr. An indwelling intravenous cannula was inserted into an antecubital vein of each subject on two occasions, with the subjects supine after fasting for 14 h. Blood (12 ml) was sampled for plasma catecholamines and serum prolactin, LH and FSH on each occasion, 1 and 5 h after cannula insertion and before administration of LH-RF. Subjects remained supine during this period except for a 5-min period of standing, 2 h after cannula insertion. LH-RF, 100 µg, was administered intravenously as a bolus and blood was sampled further, 5, 20, 60 and 120 min later. On the first occasion, bromocriptine had been taken orally 2.5 mg daily for 3 d, then 2.5 mg q12 h for another 4 d. A final dose of bromocriptine was administered 1 h after cannula insertion, that is, 4 h before LH-RF. On the second occasion, no bromocriptine had been taken for 2 weeks and none was administered during that part of the study. This allowed comparison of the plasma catecholamine response to LH-RF in the presence and absence of bromocriptine. Blood (2 ml) for catecholamine assay was transferred to tubes containing 4 mg of dithiothreitol and 20 µl of EGTA solution, 100 mg ml⁻¹. Plasma was separated and stored at -70°C until assay for catecholamines⁵. a, Dopamine; b, noradrenaline; c, adrenaline. * $P < 0.01$, significantly different from basal level. ** $P < 0.001$.

might act on presynaptic receptors of peripheral catecholaminergic neurones to decrease plasma catecholamines. It might act on dopamine receptors¹¹ or α -adrenoreceptors¹² to inhibit noradrenaline release from noradrenergic neurones as we discussed for bromocriptine⁷. It might also decrease dopamine release from peripheral dopaminergic or noradrenergic neurones. LH-RF-induced depression of adrenaline release, most of which probably comes from the adrenal medulla, is more difficult to explain by a peripheral rather than a central mechanism. If the LH-RF effect on plasma adrenaline is centrally mediated, it seems more probable that the effects on plasma dopamine and noradrenaline are also mediated centrally rather than peripherally.

Further support for the suggestion that LH-RF may decrease plasma catecholamines by acting directly on presynaptic receptors of catecholaminergic neurones is provided by our observations that bromocriptine prevents the LH-RF-induced depression of plasma catecholamines. Bromocriptine interfered with this effect of LH-RF at a time when its own effect on plasma catecholamines had ended. However, as the effect of bromocriptine on serum prolactin levels persisted at this time, it is possible that it still occupied dopamine receptors.

Further evidence that LH-RF may act on central catecholaminergic neurones is provided by our observations in rats that LH-RF decreased hypothalamic dopamine- β -hydroxylase activity (G.R.V.L. and R. Mascardo, unpublished).

In addition to its well known effects on serum LH and FSH, LH-RF increased serum prolactin in our normal men (Table 1). Such an effect might be mediated directly on anterior pituitary

Table 1 Serum prolactin, LH and FSH responses to LH-RF in the absence and presence of bromocriptine

Time (min)	Prolactin (ng ml ⁻¹)*		LH (mU ml ⁻¹)*		FSH (mU ml ⁻¹)*	
	Control	Bromocriptine†	Control	Bromocriptine†	Control	Bromocriptine†
Pre-LH-RF‡	3.9±0.2	<2.0	2.2±0.2	2.3±0.2	1.9±0.2	2.1±0.2
LH-RF§						
+20	5.7±0.6	<2.0	13.9±0.9	13.6±3.5	4.6±0.4	3.1±0.2
+60	5.2±0.7	<2.0	10.4±1.9	12.9±2.6	3.6±0.1	3.6±0.7
+120	4.0±0.3	<2.0	6.6±1.1	7.2±1.3	3.2±1.0	3.7±0.4

* Serum prolactin, LH and FSH were assayed by standard radioimmunoassay methods described elsewhere. All values are concentration means ± s.e.m.

† Bromocriptine 2.5 mg administered orally 240 min before LH-RF.

‡ All Pre-LH-RF values are pooled means of 18 determinations, six samples taken at intervals during the 4 h before LH-RF administration from each of the three subjects.

§ LH-RF, 100 µg, was administered as an intravenous bolus.

|| Significantly different from Pre-LH-RF concentration at $P < 0.05$ using analysis of variance and Duncan's multiple range test.

cells or indirectly in the median eminence. If LH-RF inhibits dopamine release from tuberoinfundibular dopaminergic neurones, as speculated above for catecholamine neurones generally, and dopamine acts to inhibit prolactin secretion, then increased prolactin secretion could be expected following LH-RF administration. Bromocriptine prevented this LH-RF-induced increase in serum prolactin, providing another parallel with the interference of LH-RF-induced effects on plasma catecholamines. There were no apparent effects of bromocriptine on the plasma LH and FSH responses to LH-RF.

If the effects of LH-RF on plasma catecholamine concentrations are mediated through presynaptic receptors as suggested above then the presynaptic receptor mechanisms involved must be functionally intact to produce a normal response. Thus, the plasma catecholamine response to LH-RF may provide an index of normal function of certain catecholaminergic neurones in man. As a corollary, dysfunction of certain catecholamine neurones might be demonstrated by abnormal plasma catecholamine responses to LH-RF. Support for this hypothesis is provided by the demonstration that in contrast to normal men, patients with acromegaly show no changes in plasma dopamine, noradrenaline or adrenaline following LH-RF administration¹³. Thus, acromegaly may represent a disorder of catecholaminergic neurones. Furthermore, the plasma catecholamine response to LH-RF may prove useful as a test of catecholaminergic dysfunction in a wide variety of disorders such as Parkinson's disease, essential hypertension, autonomic neuropathies and poorly defined hypothalamic disorders.

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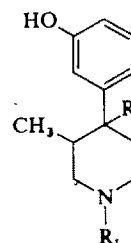
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New structural concepts for narcotic antagonists defined in a 4-phenylpiperidine series

NARCOTIC antagonists are generally considered as *N*-methylcyclopropyl or *N*-allyl (for example, nalorphine) type derivatives of *N*-methyl (for example, morphine) agonists. This type of substitution has often been considered necessary for potent narcotic antagonist activity^{1,2}. However, in the 4-phenylpiperidine series (as in meperidine) where a methyl substitution on the nitrogen has been associated with agonist activity, the substitution of an *N*-allyl derivative has not produced narcotic antagonists³⁻⁵. There are compounds, such as *L*-metazocine^{6,7} and profadol⁸, which contain a methyl-substituted basic nitrogen, and possess narcotic antagonist properties, but these are also agonists and their antagonist activity is relatively weak. During the pharmacological investigation of a new series of phenylpiperidines we discovered an *N*-methyl derivative that was a pure narcotic antagonist devoid of agonist effects. We also observed that nitrogen substituents that had increased agonist potency in the meperidine series⁹ when substituted in compounds of the present series surprisingly augmented antagonist potency to the level observed with naloxone⁴. Examination of the inhibition of specific ³H-naloxone binding of these derivatives in bovine striatal membranes demonstrated that their relative potency was due to differences in receptor interaction. We used the most potent member of the series to study its stereoselectivity. We report here the structure-activity relationships of some 1,3,4-trialkyl-4-phenylpiperidines that clearly demonstrate the importance of a 3-methyl substituent in contributing narcotic antagonist properties within the phenylpiperidine series as a whole. These compounds may well represent the first new class of pure narcotic antagonists since the discovery of naloxone.



Narcotic antagonist measurements for the 1,3,4-trialkyl-4-phenylpiperidine series are shown in Table 1. The AD₅₀ (dose that reduced the morphine response by 50%) for the *N*-methyl compound (I) was 0.24 mg per kg in rats and 1.0 mg per kg in mice. These values are comparable to the AD₅₀ values for

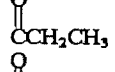
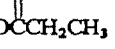
nalorphine of 0.4 mg per kg and 0.45 mg per kg in rats and mice, respectively. The allyl (II) and methylcyclopropyl (III) substitution did not increase the antagonist potency, as might have been expected in a multicyclic series (for example, morphinan⁴ and benzomorphan¹⁰). There was an increase in antagonist potency when the *N*-methyl was changed to *N*- β -phenethyl (IV) or *N*-2-propiofenone (V). Antagonist potency of the *N*-propiofenone derivative (V) was comparable to that of naloxone. Resolution of the 2-propiofenone (V) into its (+)-isomer (VII) and (-)-isomer (VI) provided only partial separation of activity, as both were antagonists, with the (+)-isomer being 2–6 times more potent than the (-)-isomer.

The 1,3,4-trialkyl-4-phenylpiperidines (Table 1) were pure antagonists as judged by the following observations. They were without measurable narcotic agonist activity (analgesia) at 100 mg per kg, given subcutaneously (s.c.), in the mouse writhing analgesic test, where compounds with mixed agonist/antagonist activity (such as nalorphine) exhibit analgesic effects (method previously described¹¹). More convincingly, in an *in vitro* measurement sensitive to narcotic agonist effects and free of the influence of metabolism, the *N*-propiofenone (V) was without measurable narcotic agonist effects. This lack of effect *in vitro* was on the twitch height of the electrically stimulated longitudinal muscle of the guinea pig ileum (method previously described¹¹). Compounds with mixed agonist/antagonist properties depress the twitch height in relation to the potency of their agonist effects, whereas pure antagonists (such as naloxone) show negligible depressant effects¹⁰. Compound V, like naloxone, lacked agonist activity at 10 μ g ml⁻¹, but completely antagonised the narcotic agonist effects of morphine at a concentration of 10 ng ml⁻¹.

The increase in antagonist potency observed when the *N*-methyl is changed to *N*-propiofenone is particularly interesting because this substitution is known to enhance agonist activity in the meperidine and prodine series⁹. Table 2 shows the receptor affinities of these 1-alkyl- β -3,4-dimethyl-4-phenylpiperidines as measured by their inhibition of ³H-naloxone binding to bovine striatal membrane homogenates in the presence and absence of NaCl. The rank order of the IC₅₀ values for compounds I–V closely parallels the observed *in vivo* antagonist potencies given in Table 1. The *N*-methyl (I), *N*-allyl (II) and *N*-methylcyclopropyl (III) derivatives all had relatively high IC₅₀ values. However, substitution of the *N*-methyl group by either the *N*- β -phenethyl (IV) or *N*-2-propiofenone (V) groups resulted in a 100-fold increase in competitive binding, comparable to that of nonradioactive naloxone. The ³H-naloxone binding of these derivatives was essentially unchanged in the presence of NaCl, confirming their binding to be that of a narcotic antagonist¹².

The importance of the methyl at position 3 on the piperidine ring is shown in Table 1. Compound IX, a reported narcotic agonist¹³ lacking the 3-methyl moiety, is devoid of narcotic antagonist activity. Table 1 also shows the structural specificity of the antagonist activity in that the 3- α -methyl isomer (VIII) (*cis*-3-methyl, 4-methyl) is about 50 times less active than the 3- β -methyl isomer (I) (*trans*-3-methyl, 4-methyl). The significance of the 3-methyl substitution is further indicated in Table 1. A methyl substituted on the position of the ketobemidone molecule provided a weak antagonist (X), which contrasts sharply with the potent agonist effects of ketobemidone¹⁴. The 3-methyl ketobemidone derivative (X) is a mixed agonist/antagonist producing inhibition of writhing (analgesia)

Table 1 1,3,4-trialkyl-4-phenylpiperidines and their narcotic antagonist activities

R ₁	R ₂	Configuration	Antagonist activity AD ₅₀ (mg per kg, s.c.)	
			Rats	Mice
I CH ₃	CH ₃	β^*	0.24	1.0
II CH ₂ CH=CH ₂	CH ₃	β	0.47	0.98
III CH ₂ — 	CH ₃	β	0.72	0.72
IV CH ₂ CH ₂ Ph	CH ₃	β	0.11	0.14
V CH ₂ CH ₂ C(=O)Ph ($\pm$ isomer)	CH ₃	β	0.056	0.049
VI CH ₂ CH ₂ C(=O)Ph (- isomer)	CH ₃	β	0.050	0.14
VII CH ₂ CH ₂ C(=O)Ph (+ isomer)	CH ₃	β	0.023	0.025
VIII CH ₃	CH ₃	$\alpha^\dagger$	33	39
IX CH ₃	CH ₃	$\rightarrow^\ddagger$	None	None
X CH ₃		β	21	38
XI CH ₃		β	6.2	9.9
Pentazocine			20	14
Nalorphine			0.40	0.45
Naloxone			0.022	0.079

Compounds I–VIII were prepared in a multiple-step procedure starting from 3-methoxyphenylacetone. The isomeric configurations were confirmed by X-ray crystallography and ¹³C-NMR analysis. Compounds IX (ref. 13), X (ref. 16) and XI (ref. 17) were synthesised by modified published procedures. Water-soluble salts were prepared in all instances. The ability of each test compound to reduce the opiate effect of morphine was measured in mice by determining the antagonism of the morphine-induced Straub tail and increased locomotion. Fasted male Cox Standard albino mice (17–21 g) were used. Morphine sulphate was administered at 40 mg per kg, s.c., and the presence or absence of the Straub tail and increased locomotion were noted and scored 15 min later. The test antagonist was administered s.c. at various doses 15 min before the morphine sulphate. The ability of the test compound to reduce or antagonise the analgesic effect of morphine was measured in the rat tail heat analgesic test (previously described¹⁰). The only change in the method was to increase the stimulus by using 7 A currents in the heating wire. We routinely measured the analgesic effect of morphine sulphate 10 min after the s.c. administration of 10 mg per kg. The elevations in reaction time following morphine were 2.5–8.6 s, with the average of 24 groups being 4.8 s. The test antagonist was administered s.c. at various doses 20 min before the morphine sulphate. The increase above controls for the morphine group was compared to the group receiving both morphine and the antagonist, and the % reduction in the morphine response determined. The AD₅₀ value, determined by "the use of the regression line in reverse"¹⁸ was the dose required for a 50% reduction in the response to morphine.

* *trans*-3-methyl, 4-R.

† *cis*-3-methyl, 4-R.

‡ Compound IX lacks the 3-methyl group.

Table 2 Inhibition of ³H-naloxone binding by 1-alkyl-β-3,4-dimethyl-4-phenylpiperidines in the presence and absence of NaCl

Compound	Concentration of compound at 50% inhibition (IC ₅₀ , nM)		Na shift (+NaCl/−NaCl)
	+NaCl	−NaCl	
I	50	100	0.5
II	60	90	0.7
III	60	60	1
IV	0.4	1.5	0.3
V	2	1	2
Naloxone	1.2	1.6	0.8
Levorphanol	60	2	30

Bovine striatal membranes of 0.6 mg protein were incubated at 24 °C for 15 min in 1 ml of 100 mM Tris-Cl, pH 7.4 buffer containing 2 nM ³H-naloxone (38 Ci mol^{−1}, NEN) and 100 mM NaCl as indicated. Samples were centrifuged at 1,085g for 10 min. Radioactivity in the pellet was determined by a liquid scintillation method. The IC₅₀ value was obtained by plotting % inhibition against a 4-decade change in concentration of each compound in a semilog plot. Specific binding of ³H-naloxone was defined as the difference in binding between samples with and without 1 μM naltrexone. In control samples, ³H-naloxone was specifically bound to bovine striatal membranes at 40.8±1.5 and 119.7±2.6 fmol per mg protein in the absence and presence of 100 mM NaCl, respectively. Other experimental conditions were followed according to published methods¹⁹.

in mice at 10 mg per kg, s.c. Hydroxylation of the phenyl in β-prodine, a compound already possessing the appropriate 3-methyl, changed this potent agonist¹⁵ into a narcotic antagonist (XI) with a greater potency than that of pentazocine, but lacking measurable agonist activity.

In summary, we report here the discovery of several novel phenylpiperidines which are potent narcotic antagonists without measurable agonist properties. We believe that this defines a new class of narcotic antagonists in which the 3 position of the piperidine ring rather than substitution at the nitrogen is the area critical for determining antagonist activity.

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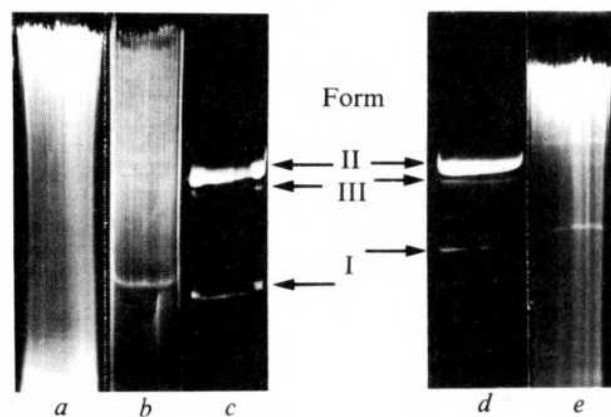
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Viral sequences related to a human skin papillomavirus in genital warts

GENITAL WARTS (condylomata acuminata) are benign tumours which are mostly venereally transmitted¹; their malignant conversion has been observed only rarely (see refs 2, 3 for reviews). Typical viral particles, long believed to be those of the human papillomavirus (HPV) found in skin warts⁴, were observed irregularly in these lesions^{5–7}. Epidemiological¹ and serological⁶ data, as well as molecular hybridisation experiments showing the absence of DNA sequences homologous to skin HPV DNA in condylomas^{8,9}, have raised the possibility that these lesions are due to a distinct HPV. However, the demonstration of at least four distinct HPV types in skin lesions^{10–13} requires a reexamination of this problem. We have identified four types of HPV showing little, if any, DNA sequence homology and distinct serological properties: HPV type 1 (HPV-1) associated with deep plantar warts¹², HPV type 2 (HPV-2) isolated from common warts¹², HPV type 3 (HPV-3) found in flat warts and in flat wart-like lesions of patients suffering from epidermodysplasia verruciformis (EV)¹³ and HPV type 4 (HPV-4) isolated from morphologically distinct EV lesions (reddish or pityriasis versicolor-like plaques)¹³. HPV-4 is found more frequently than HPV-3 in patients with EV showing a malignant conversion of some lesions^{13,22}. We report here RNA–DNA hybridisation data indicating that genital warts are associated with an HPV showing little, if any, DNA sequence homology with HPV-1, HPV-2 and HPV-3 and a low extent of homology with an HPV-4 isolate taken as prototype in previous studies¹³.

Fig. 1 Agarose gel electrophoresis of DNAs extracted from genital warts. Warts obtained from seven patients (Table 1) were processed according to a modified Hirt method^{12,14}. Minced tissues (0.3–1 g) were incubated in 10 ml of 0.01 M Tris, 0.01 M NaCl, 0.01 M EDTA, 0.5% SDS containing 50 μg ml^{−1} of proteinase K (Merck). After a 6 to 10 h incubation at 37 °C, NaCl concentration was adjusted to 1 M, and the mixtures were kept at 4 °C overnight. Supernatants were extracted twice with phenol and DNAs were ethanol-precipitated and redissolved in 0.5–1 ml of 0.01 M Tris, 0.001 M EDTA, pH 7.9. Yields of DNA ranged from 0.04 to 1 mg as determined by absorbance at 260 nm. DNAs (30–300 μg per slot) were fractionated by electrophoresis in vertical 0.8% agarose slab gels (0.04 M Tris, 0.02 M ammonium acetate, 0.002 M EDTA, pH 7.8) for 20 h at 30 mA, taking HPV-1 form I, II and III DNA as standards (10 μg DNA per slot). Gels were stained in 5 μg ml^{−1} ethidium bromide and electropherograms visualised under UV light, as shown for the DNAs extracted from genital warts of patients N.M. (a), W.K. (b) and M.B. (c) and for HPV-1 form I, II and III DNA (c, d). The slower migration of the genital wart HPV DNA is probably due to the high viscosity of the samples.



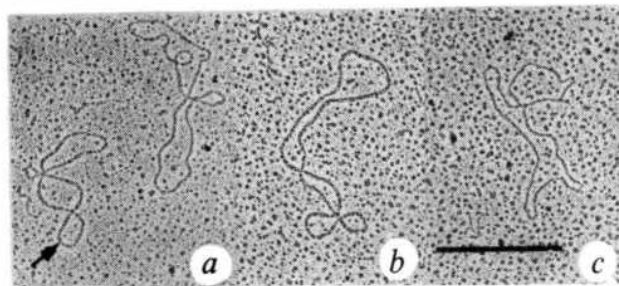


Fig. 2 Electron micrographs of circular HPV DNA molecules obtained from genital warts. DNAs were selectively extracted from lesions of patients W.K. (a), W.L. (b) and M.B. (c) and further purified by Agarose gel electrophoresis as described in the legend to Fig. 1. Bands with a mobility comparable to that of HPV-1 form I DNA molecules were sliced from the gel and DNA was eluted electrophoretically, mounted on collodion-coated grids, Pt-Pd shadowed and observed with a Siemens Elmiskop 101 electron microscope¹². The arrow indicates a molecule of replicative form of Φ X174 phage DNA (Φ X174 RF DNA), taken as length standard. The bar represents 0.5 μ m.

The present study was carried out with genital warts obtained from seven patients (Table 1). Because the low virus content of lesions^{5,7,9} precluded virus purification, viral DNA was extracted selectively from the tissues^{12,14} and purified by Agarose gel electrophoresis¹⁵ (Fig. 1). Four of the DNA preparations did not yield detectable bands (Fig. 1a). However, preparations obtained from patients W.K., W.L. and M.B. showed bands migrating slightly more slowly than those of HPV-1 form I, II or III DNA (Fig. 1b-e). Circular DNA molecules of about 2.5 μ m, typical of HPV DNAs¹⁶ (Fig. 2), were observed in electrophoretic eluates of the bands corresponding to form I DNA. This result is in contrast to the previously reported failure to demonstrate the presence of a distinct papova-like circular DNA in condylomata acuminata⁹. Comparable length distributions were found for W.K. and M.B. HPV DNAs purified by Agarose gel electrophoresis (Fig. 3a, c) and for W.L. HPV

DNA molecules found in total DNA (Hirt supernatant) (Fig. 3b). The lengths of W.K., M.B. and W.L. HPV DNAs were 1.493 ± 0.08 , 1.439 ± 0.07 and 1.462 ± 0.08 Φ X174 RF DNA units, corresponding to 8,025, 7,735 and 7,860 base pairs, respectively. Molecular weights inferred from these values ($5.2-5.3 \times 10^6$) are close to those determined for the DNAs of the HPVs so far identified^{12,13}. A second population of circular DNA molecules, about 5.1 μ m long, was observed in the total W.L. DNA preparation (Fig. 3b). This could represent either HPV DNA dimers or, more probably, mitochondrial DNA molecules^{9,18}.

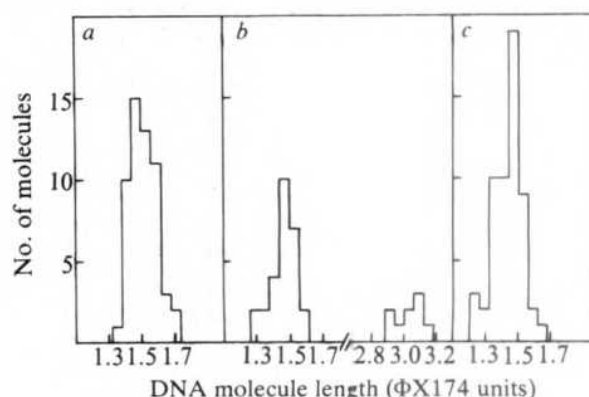


Fig. 3 Histograms of the lengths of genital wart HPV DNA molecules. DNA selectively extracted from lesions (b, W.L.) and further purified by Agarose gel electrophoresis (a, W.K.; c, M.B.) (see legend to Fig. 2) were mixed with Φ X174 RF DNA molecules. Contour lengths of relaxed circular molecules were measured on electron micrographs and expressed as fractional length of Φ X174 RF DNA¹².

Total DNA from genital warts, as well as purified form I DNA preparations (W.K. and M.B. HPV DNAs), were hybridised with RNAs complementary (cRNAs) to HPV-1, HPV-2, HPV-3 or HPV-4 DNAs^{12,13}. In these conditions^{12,13,20}, 50-60% of the radioactivity bound to the filters after hybridisation of

Table 1 Search for DNA sequences homologous to skin HPV DNAs in DNA extracted from genital warts

Patients	Location of lesions	DNA on filters	Radioactivity bound on filters (%) after hybridisation with labelled RNAs complementary to the DNAs of			
			HPV-1	HPV-2	HPV-3	HPV-4
W.K. (M, 28)	Meatus	Total	0.1	0.5	0.4	7.3
		Eluate	-0.3	0.1	-0.3	8.8
M.B. (M, 26)	Glans, prepuce	Total	0.1	-0.3	-0.4	4.2
		Eluate	0.3	0.2	0.1	5.1
W.L. (M, 26)	Prepuce	Total	0.1	0.9	1.1	4.9
N.M. (M, 44)	Meatus, prepuce	Total	0.3	0.1	1.1	3.6
W.B. (M, 64)	Prepuce	Total	0.2	1.0	0.8	5.5
B.B. (M, 30)	Groin, anus	Total	0.5	0.4	0.5	3.5
M.K. (F, 24)	Vulva	Total	0.5	0.3	0.3	5.0
		HPV-1	55.9	0.3	0.1	0.3
		HPV-2	0.2	58.3	0.5	0.1
		HPV-3	0.7	0.3	49.2	0.2
		HPV-4	0.5	-0.2	-0.1	52.5
		Thymus	0.1	0.3	0.1	0.8
		Liver	0.2	0.1	1.0	0.4

RNA-DNA filter hybridisation under paraffin was carried out as described by Kourilsky *et al.*²⁰ with triated RNAs (specific activity, $3-4 \times 10^7$ c.p.m. μ g⁻¹) complementary (cRNAs) to the four HPV DNAs transcribed *in vitro* as previously described¹². HPV DNAs were obtained from a single plantar wart (HPV-1)¹², pooled hand common warts of a single patient (HPV-2)¹², and pooled scrapings of lesions of two patients with E.V. (HPV-3, HPV-4)¹³; these HPV-2, HPV-3 and HPV-4 preparations were those taken as prototypes in previous studies^{12,13}. Genital wart DNAs were selectively extracted from the lesions of seven patients according to a modified Hirt procedure^{12,14} (total DNA) or further purified by Agarose gel electrophoresis (eluate), as described in the legends to Figs 1 and 2. Nitrocellulose filters (two per assay) carrying heat-denatured prototypical HPV DNAs, W.K. and M.B. HPV DNAs (about 50 ng, as determined with Φ X174 RF DNA as a standard²¹), genital wart DNAs (2.5-7.5 μ g), human fetal thymus or liver DNA (7.5 μ g), as well as blank filters, were added with 4 μ l of cRNA (2,000 c.p.m. per filter) in 0.30 M NaCl, 0.030 M sodium citrate-50% formamide. Heat-denatured fetal human thymus DNA was added to the hybridisation mixture (0.5 μ g per assay) to eliminate any hybridisation to cellular DNA on filters. Paraffin was added and filters were incubated for 15 h at 37 °C, processed and counted as previously described¹². Sex and age (yr) of patients are given in parentheses.

homologous viral DNAs and cRNAs, whereas no radioactivity was retained using heterologous prototypical cRNAs and DNAs (Table 1). No significant hybridisation was obtained with any of the genital wart DNAs (total or form I) and HPV-1, HPV-2 or HPV-3 cRNAs. However, all the total DNA preparations, as well as purified form I DNA from W.K. and M.B. tumours, showed significant levels (3.5–8.8%) of hybridisation with the HPV-4 cRNA probe (Table 1).

Our results indicate that three known types of human papillomavirus, HPV-1, HPV-2 and HPV-3, are not generally associated with genital warts (see, however, ref. 22). On the other hand, we find that viral sequences related to a fourth type, HPV-4, are present in DNA of the tumours studied here. The present data do not, however, show to what extent the sequences are related, and this remains a subject for further study.

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An insert in the single gene for the large ribosomal RNA in yeast mitochondrial DNA

MITOCHONDRIAL DNA (mtDNA) contains genes for the ribosomal RNAs (rRNAs) and transfer RNAs (tRNAs) used in mitochondrial protein synthesis and for a limited number of proteins of the mitochondrial inner membrane^{1–4}. By a combination of genetic and physical mapping, the location of most of these genes on yeast mtDNA has been determined^{3,4}. There is a single gene for the RNA from the large subunit of

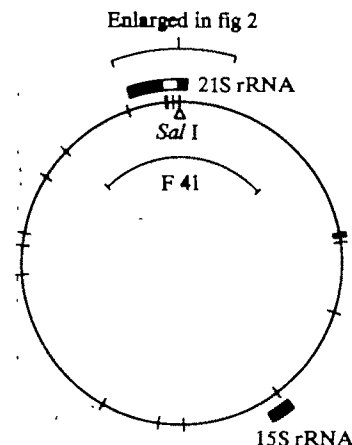


Fig. 1 The physical map of mtDNA from *S. cerevisiae* strain JS1-3D, showing the position of the rRNA genes and the recognition sites for endonucleases *HindII* and *HindIII* and the single site for endonuclease *SalI* (from refs 5, 10). The contour length is 72,000 base pairs. The region of the map enlarged in Fig. 2 and the segment of mtDNA retained in petite mutant F41 are indicated.

yeast mitochondrial ribosomes⁵, but, nevertheless, this RNA hybridises to non-adjacent fragments on the physical map of mtDNA⁶. Although this suggested that the gene for this RNA is split, like some eukaryotic nuclear genes⁷, the presence of partial gene duplications complicated the interpretation of our hybridisation experiments⁶. By electron microscopy of RNA-DNA hybrids we now present direct evidence for an insertion in this rRNA gene.

Figure 1 shows a simplified map of yeast mtDNA, and Fig. 2 gives a more detailed map of the region of the large rRNA cistron. The rRNA hybridises to the *HindII* and *HindIII* fragments TD9, DT14 and TT1 (hatched in Fig. 2), but not to TD15 and the small 70-base pair fragment next to it⁶. Additional hybridisation experiments (unpublished) have shown that this is not due merely to partial gene duplication, because no homology was found between sequences hybridising with 21S rRNA in fragment TT1 and fragment TD9.

To prove that the rRNA gene is indeed split, hybrids were made between rRNA and the *HaeIII* fragment indicated in Fig. 2. The rRNA was obtained by fractionating total mtRNA from *Saccharomyces cerevisiae* strain JS1-3D by agarose gel electrophoresis and eluting the 21S rRNA band 8. The *HaeIII* fragment was isolated⁹ from the mtDNA of petite mutant F41. This petite has been derived from *S. cerevisiae* strain JS1-3D; it contains the segment of wild-type mtDNA indicated in Fig. 1 which has been shown by restriction enzyme digestion and hybridisation analysis^{6,10} to be identical to the corresponding wild-type sequence. Hybrids were formed in high formamide concentration to avoid DNA renaturation¹¹, and spread in conditions which allow a clear distinction between single-stranded RNA (kinky) and DNA (smooth) strands. Figure 3 shows two of the predominant hybrid molecules present in electron micrographs of these hybrids and the insert gives our interpretation of these molecules. The single-stranded DNA loop between two duplex hybrid sections proves that the mature rRNA is complementary to non-adjacent segments of mtDNA. Not a single full-size hybrid molecule without this loop was observed. The left-right orientation of the hybrid molecules relative to the map in Fig. 2 is determined by the morphology of the single-stranded ends, which shows that the left-hand end is RNA and the right-hand end DNA. This orientation was confirmed by the presence of rare hybrid molecules that had reannealed with a broken complementary DNA strand of the *HaeIII* fragment, resulting in hybrids with a partially duplex end. This duplex end was present at the side of the short hybrid segment (segment c in Fig. 3).

Table 1 Size of hybrid and single-stranded DNA regions in 42 rRNA hybrid molecules

Segment (see Fig. 3)	$\mu\text{m} \pm \text{s.d.}$	Size Base pairs
a	0.33 ± 0.02	830
c	0.20 ± 0.01	510
b	0.50 ± 0.03	1,160

Measurements of hybrid sections were calibrated with the circular replicative form of phage ΦX174 (5,375 base pairs²⁰), co-spread and measured on the same grid. Single-stranded DNA segments were calibrated using the single-stranded *HaeIII* fragment (4,300 nucleotides) present on the same grid.

The position of the rRNA gene on the map in Fig. 2 follows from the quantitative information on 42 hybrid molecules summarised in Table 1 and from the sections of the DNA previously shown to hybridise with rRNA^{6,10}. The location of the insert is determined by the size of section a (830 base pairs) and the position of the left-hand end of the *HaeIII* fragment¹², which we have mapped by double-digestion analysis. The size of the insert, determined from the size of loop b, is 1,160 base pairs. From the size of sections a–c, we infer that the rRNA cistron runs about 400–500 base pairs into fragment TT1.

Although inserts have previously been found in the nuclear rRNA genes of *Drosophila*^{11,13–16}, it has not yet been possible to decide if these split genes yield mature rRNA. With yeast mtDNA this is certain, because no other gene is present.

The region of the 21S rRNA on yeast mtDNA contains interesting structural and genetic elements, as indicated in Fig. 2. First, there is the 1,050-base pair insertion VI of Sanders *et al.*⁵ found by us^{5,6} and by Jacq *et al.*¹² in the ω^+ strains and lacking in the ω^- strains characterised by Slonimski *et al.*¹⁷. This 1,050-base pair insertion coincides with the intervening sequence in the rRNA gene within the limits of error of our measurements. Other interesting genetic loci are *rib-1* (chloramphenicol resistance) and *rib-2* (erythromycin resistance). We have argued previously¹⁸ that mutations at these loci affect the mitochondrial ribosome by altering the nucleotide

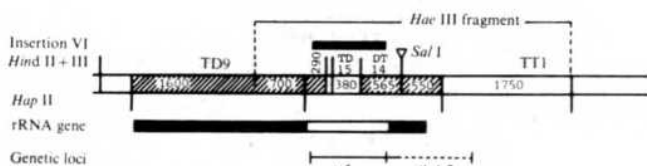


Fig. 2 The physical map of the segment of yeast mtDNA that contains the gene for the large 21S rRNA. The position of the cleavage sites for endonucleases *HindII*, *HindIII*, *HapII* and *SalI* on the mtDNA of *S. cerevisiae* strain JS1-3D are taken from refs 5, 6 and 10. Hatched fragments hybridise with 21S rRNA^{6,10}. The position on the map of the *HaeIII* fragment, used for electron microscopy of DNA–RNA hybrids, was determined by double-digestion analysis with endonucleases *SalI*, *HindII* and *HindIII* (unpublished). The position of the rRNA gene was deduced from the hybridisation results^{6,10} and the measurements in Table 1; from the size of the rRNA (3,700 nucleotides²¹) we infer that the left border of the rRNA gene must be close to the left *HapII* site in TD9, as drawn, but this has not been verified. The positions of the genetic loci are taken from ref. 10 and unpublished results; ω is a locus which affects the recombination of markers around it; there is a one-to-one correlation^{6,12} between the ω^+ allele and insertion VI of Sanders *et al.*⁵. Mutations at *rib-1* and *rib-2* confer insertion of mitochondrial protein synthesis to chloramphenicol and erythromycin, respectively. *rib-1* and *rib-2* are most probably located in the region indicated by the continuous line, but a position more to the right, indicated by the broken line, is not excluded.

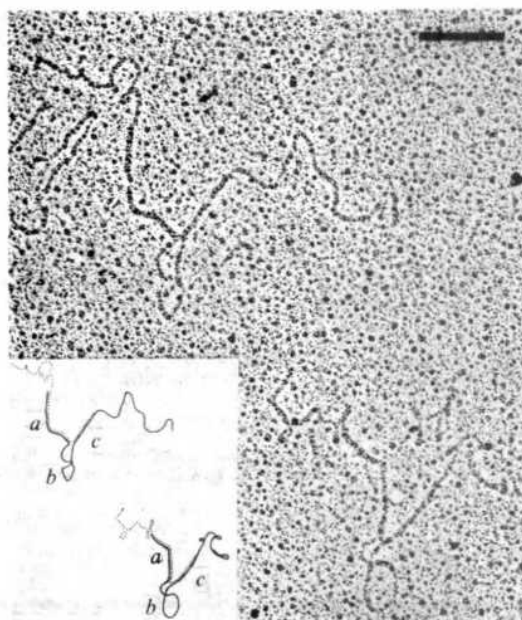


Fig. 3 Electron micrograph of RNA–DNA hybrids made between 21S rRNA and the denatured *HaeIII* fragment indicated in Fig. 2. The hybridisation and visualisation of hybrids was done by the procedure of Wellauer and Dawid¹¹ as described²², with the modification that the hybridisation was carried out for 2 h at 40 °C. Main figure electron micrograph showing two adjacent hybrid molecules; scale bar, 0.2 μm . Inset, interpretation of main figure; dotted lines are RNA, straight lines DNA.

sequence of rRNA. The precise mapping of these loci, on the one hand, and of the rRNA, on the other hand, now lends support to this suggestion.

In previous papers^{4,6,19} from our laboratory, it was suggested that the genes for subunit I of cytochrome *c*-oxidase (*oxi-3* locus) and for cytochrome *b* (*cob* locus) are split to account for the discrepancy between apparent locus size and protein product. Our present results re-emphasise this suggestion. Yeast mitochondria with their simple and well-characterised genome⁴ may well turn out to be an attractive experimental system for the study of the nature and evolution of gene inserts and the splicing enzymes required to process the primary transcripts of such genes.

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Yeast has a true stringent response

IN bacteria there is a coordinate regulation of the synthesis of ribosomal RNA and ribosomal protein¹. This is most evident from studies of the rates of synthesis of ribosomal components as a function of the growth rate of the cell^{2,3}. Another example is the 'stringent response', in which cells deprived of an essential amino acid shut down the transcription of ribosomal RNA as well as that of messenger RNA for ribosomal proteins^{4,5}, but increase the synthesis of mRNA for certain biosynthetic proteins⁶. Although this stringent response is at least partially due

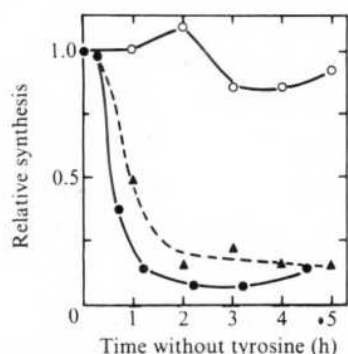


Fig. 1 Macromolecular synthesis in cells deprived of tyrosine. Protein synthesis (●): *Saccharomyces cerevisiae*, strain A364A, a, *ade*⁻, *ura*⁻, *his*⁻, *lys*⁻, *try*⁻, were cultured in synthetic complete medium at 23 °C as described elsewhere⁷, to a concentration of 1×10^7 . The cells were collected by filtration, washed and suspended in medium lacking tyrosine. At various times 5 ml of culture was added to a flask containing 2.5 μ Ci of ¹⁴C leucine (120 μ Ci μ mol⁻¹). At 5-min intervals for 30 min, 0.5 ml of culture was transferred to 1 ml 10% trichloroacetic acid (TCA), boiled briefly and the acid insoluble radioactivity determined. The rate of incorporation was determined from the slope of the resultant curves compared with the rate of incorporation of the culture before the medium change, and plotted at the midpoint of the 30-min incorporation. Ribosomal RNA transcription (▲): cells were deprived of tyrosine as described above. At intervals, a 3-ml aliquot was mixed with 0.5 mCi of ³H (methyl) methionine. After 60 s the culture was chilled and the RNA extracted from broken cells and analysed on 2.75% polyacrylamide gels as described⁷. The radioactivity in RNA species was normalised to the absorbance of 25S RNA and compared with the value for a culture labelled before the medium change. Template activity (○): cells were deprived of tyrosine as described above. At intervals, 25-ml aliquots were chilled and total RNA prepared. The template activity of each preparation was determined at limiting RNA concentration¹², and compared with the activity for the RNA from the culture before the medium change.

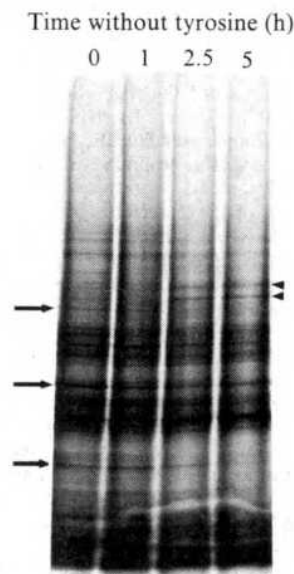
to the *rc* gene product and/or its synthesis of ppGpp, little is known about the detailed mechanisms involved in controlling the two types of transcription. To what degree does the stringent response exist in eukaryotic cells, which use separate polymerases for the transcription of ribosomal RNA and messenger RNA^{6,7}? We⁷ and others⁸ have shown that in *Saccharomyces cerevisiae* deprived of an amino acid, the transcription of ribosomal RNA is severely inhibited. Ribosomal RNA transcription is also inhibited in cells treated with cycloheximide^{7,9}, but it continues almost normally in cells in which synthesis of ribosomal proteins has specifically been inhibited through a temperature-sensitive mutation¹⁰. We demonstrate here that in cells deprived of an amino acid there is a specific depletion of messenger RNA coding for ribosomal proteins.

The experiments are based on our ability to translate yeast mRNA quantitatively in a wheatgerm extract and to isolate the yeast ribosomal proteins produced¹¹. Total protein synthesis and the synthesis of yeast ribosomal proteins are linearly dependent on added yeast RNA, reaching a maximum at the same RNA concentration¹². Thus, unless a saturating concentration of yeast RNA is added to the wheatgerm extract, the radioactivity in any individual ribosomal protein spot is a measure of the relative amount of mRNA specific for that protein.

Cells were deprived of tyrosine as described in Fig. 1 and at intervals the rates of protein synthesis and ribosomal RNA transcription were determined. The effects of tyrosine deprivation on protein synthesis are complete only after an hour, presumably due to a substantial pool of tyrosine within the cells. The transcription of ribosomal RNA decreases slightly later than protein synthesis and levels off at a rate 15–20% of normal. On the other hand, the template activity of RNA prepared from cells deprived of tyrosine remains almost unchanged for 5 h (Fig. 1). As the half life of mRNA in yeast is between 6 and 30 min at 23 °C (ref. 13), substantial synthesis of new mRNA must continue, as was demonstrated directly elsewhere⁷.

Figure 2 is a one-dimensional polyacrylamide gel analysis of proteins synthesised by a wheatgerm extract in response to RNA from cells deprived of tyrosine. Although most proteins show

Fig. 2 Template activity of RNA from cells deprived of tyrosine. Cell-free translation was carried out with wheatgerm extract using as template RNA from cells deprived of tyrosine for increasing time, as described in Fig. 1. An aliquot was treated with 5% TCA, the precipitate electrophoresed on a 7.5–25% acrylamide gel in the presence of SDS according to the method of Laemmli¹⁴. Protein synthesis inhibition is indicated by the arrows, enhancement by arrowheads.



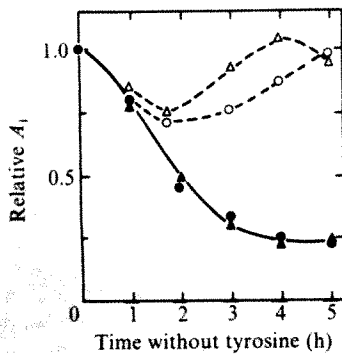


Fig. 3 Template activity for specific proteins. Aliquots of the cell-free translation described in Fig. 1 were subjected to two-dimensional polyacrylamide gel electrophoresis¹¹, and ribosomal and nonribosomal protein spots were cut out. The $A_1 = (^{35}\text{S}/^3\text{H})_i / (^{35}\text{S}/^3\text{H})_{\text{total}}$ (ref. 11) was determined for each, and compared with the unstarved control. ●, Ribosomal protein no. 5; ▲, ribosomal protein no. 33; ○, nonribosomal protein C; △, nonribosomal protein D.

little change, the synthesis of some is inhibited (arrows) and that of others enhanced (arrowheads). Figure 3 shows a quantitative analysis of the template activity for individual ribosomal and nonribosomal proteins for the experiment described in Fig. 1. It is clear that as cells are deprived of tyrosine, they gradually lose mRNA for these two ribosomal proteins. That this is true of most, but not all, ribosomal proteins is shown in Table 1. Some of the exceptions, such as nos 12 and 14, are also exempt from the coordinate regulation in the *ts* mutants described above¹¹. The values for individual proteins in Table 1 and in other experiments are remarkably uniform between experiments, suggesting that the variation between proteins is due to slight differences in the stability of mRNA and/or regulation of its transcription. Nevertheless, the ribosomal proteins seem to be regulated as a class.

Table 1 Template activity for ribosomal and nonribosomal proteins

Protein no.	Subunit	Relative template activity at 5 h	
Ribosomal proteins		Expt 1	Expt 2
1	L	0.26	0.36
2	L	0.35	0.42
5	S	0.34	0.25
6	L	0.30	0.30
8	L	0.41	0.40
11	L	0.65	0.42
12	S	1.30	0.71
13	S	0.52	0.42
14	S	0.89	0.97
24	L	0.47	0.39
28	L	0.30	0.33
29	L	0.30	0.34
30	S	0.90	0.81
33	L	0.22	0.26
38	L	0.43	0.31
39	L	0.47	0.41
40	S	0.96	0.75
41	S	0.34	0.36
45	L	0.33	0.30
46	L	0.35	0.32
Nonribosomal proteins			
C		0.96	0.98
D		0.82	0.94
E		1.06	0.92
G		1.00	1.26

Template activity for ribosomal proteins and nonribosomal proteins was measured as described in Fig. 3. The values represent the ratio of template activity of RNA from cells deprived of tyrosine for 5 h to the template activity of RNA from unstained cells, for two independent experiments. Experiment 2 is that of Fig. 3. L is the large (60S) subunit, S is the small (40S) subunit.

These results show that in yeast cells deprived of an amino acid there is a parallel inhibition in the synthesis of both ribosomal RNA and mRNA for most ribosomal proteins. The fact that the decline in mRNA activity occurs somewhat later than that of rRNA transcription may be due to the finite half life of mRNA for ribosomal proteins, which at 23 °C is probably 20–30 min^{13,15}. The kinetics in Figs 1 and 3 are consistent with the transcription of rRNA and mRNA for ribosomal proteins being inhibited simultaneously. It seems, therefore, that yeast has a fully coordinated stringent response to the deprivation of an amino acid, which involves ribosomal RNA as well as mRNA for ribosomal proteins, but excludes both tRNA and mRNA for the bulk of the cell's proteins⁷. Although ppGpp has not been detected in such conditions (J. Gallant, personal communication), a search for 'relaxed' mutants and for a regulatory molecule may be useful.

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In vivo aminoacylation and 'processing' of turnip yellow mosaic virus RNA in *Xenopus laevis* oocytes

IT is now well established that several plant and animal virus RNAs possess a 'tRNA-like' structure, as they are recognised *in vitro* by many tRNA-specific enzymes. To approach the problem of the possible physiological significance of these structures for the development of the virus, it seemed important to determine whether they are also recognised *in vivo*. The most logical approach would have been to use plant protoplast systems to study the behaviour of viral genomes in the infected cells. However, because such systems are still difficult to control, we decided to use the oocyte system. We describe here how turnip yellow mosaic virus (TYMV) RNA, which can be aminoacylated *in vitro* by valine¹, was microinjected into *Xenopus laevis* oocytes, incubated in the presence of ³H-valine and the RNA charged *in vivo* analysed. We conclude that TYMV RNA, the 3' terminal sequence of which is –CC (refs 2, 3), is aminoacylated *in vivo*. This indicates that the viral RNA is recognised by the oocyte tRNA nucleotidyltransferase and valyl-tRNA synthetase. Furthermore, the TYMV Val-RNA recovered after the *in vivo* reaction is fragmented and migrates between 4S and 5S RNA markers. This implies that (an) RNase(s) split(s) the viral genome, liberating this tRNA-like structure.

Table 1 Aminoacylation of total TYMV RNA, of TYMV coat protein mRNA or of *E. coli* tRNA^{Val} microinjected into *X. laevis* oocytes

Expt	(t)RNA injected	Species	pmol* per oocyte	No. of oocytes injected	c.p.m. per oocyte
1	None		—	40	1,546
	Total TYMV RNA		0.37	40	1,743
	<i>E. coli</i> tRNA ^{Val}		22	7	30,920
2	None		—	51	1,396
	Total TYMV RNA		0.37	53	1,509
	TYMV coat protein mRNA		0.17	56	1,130

TYMV was extracted from infected Chinese cabbage leaves according to Leberman¹². Total TYMV RNA was recovered using the phenol-chloroform-sodium laurylsulphate method^{13,14}. Partially purified viral RNA coding *in vitro* for coat protein (coat protein mRNA) was separated from genomic RNA by chromatography on a Sepharose 6B column. Fully grown oocytes from *X. laevis* females were preincubated for 3 h at 20°C in modified Barth's medium¹⁵ (100 µl per 10 oocytes) containing ³H-valine (31 Ci mmol⁻¹; Amersham) at a final concentration of 1 mCi per ml. They were microinjected¹⁶ with the following substrates in 50 nl of sterile water: 11.2 mg ml⁻¹ of *E. coli* tRNA^{Val} (Sigma), 15.4 mg ml⁻¹ of total TYMV RNA, or 870 µg ml⁻¹ of TYMV coat protein mRNA, and incubated at 20°C in fresh medium containing ³H-valine as above. After 2 and 24 h of incubation for experiments 1 and 2, respectively, the oocytes were washed with modified Barth's medium and stored at -70°C. They were homogenised and the RNA extracted as reported by Gatica *et al.*¹⁷. After ethanol precipitation, the RNA pellets were dissolved in sterile water and stored at -20°C. Aliquots were precipitated by 10% cold TCA, filtered through nitrocellulose membranes and the radioactivity retained measured in the presence of Bray's solution. Further analyses were carried out using the samples from experiment 2, except for the *E. coli* ³H-Val-tRNA^{Val}, which was from experiment 1.

*The pmol of (t)RNA injected are based on molecular weights of 2×10^6 for total TYMV RNA, 0.25×10^6 for TYMV coat protein mRNA and 25,000 for tRNA^{Val}.

To see if TYMV RNA could be charged *in vivo*, *X. laevis* oocytes were microinjected with total TYMV RNA (containing genomic RNA and coat protein mRNA)⁴, partially purified TYMV coat protein mRNA or *Escherichia coli* tRNA^{Val}. After 2 or 24 h of incubation in the presence of ³H-valine, the RNA was extracted from the oocytes and an aliquot used to determine the cold trichloroacetic acid (TCA)-precipitable counts; these are given in Table 1. No significant variation in counts is observed with oocytes injected with total TYMV RNA as compared to control, uninjected oocytes, even after 24 h of incubation. The same is true when TYMV coat protein mRNA is injected. Conversely, incubation with *E. coli* tRNA^{Val} leads to a 20-fold stimulation after only 2 h of incubation.

The fact that no increase in cold TCA-precipitable counts could be detected with the viral RNA preparations can certainly be explained by the low amount of TYMV RNA injected as compared to tRNA^{Val}. Indeed, the charging capacity of total TYMV RNA on a weight basis is about 80 times lower than that of a tRNA (see legend to Table 1).

Because it had not been possible to detect TYMV RNA charging by TCA precipitation, it was necessary to analyse the size of the ³H-Val-oligonucleotides recovered after T₁ RNase digestion of the RNA extracted from the oocytes. This was carried out by paper electrophoresis in 0.5 M formic acid (results not shown) or by thin-layer chromatography (TLC) on polyethyleneimine-cellulose (PEI-cellulose) plates (Fig. 1).

The T₁ digests of TYMV Val-RNA charged *in vitro* reveal the presence of a Val-pentanucleotide (Fig. 1, sample 4) and those of *E. coli* Val-tRNA, also charged *in vitro*, of a Val-pentanucleotide and a Val-tredecannucleotide (sample 6). This agrees with the known sequence of the 3' region of TYMV RNA^{2,5,6} and of the valine-specific *E. coli* tRNAs^{7,8}, respectively.

The endogenous oocyte tRNAs charged *in vivo* (sample 1, see also samples 2 and 3) produce two Val-oligonucleotides of about eight and nine residues, plus a third spot which migrates faster than a pentanucleotide.

The analyses of the Val-oligonucleotides obtained after T₁ digestion of the RNAs extracted from oocytes injected either by total TYMV RNA (sample 2) or TYMV coat protein mRNA (sample 3) showed in each case the presence of a Val-pentanucleotide, demonstrating the *in vivo* aminoacylation of TYMV RNA. This ³H-Val-pentanucleotide is much less intense when the oocytes are injected with TYMV coat protein mRNA than with total TYMV RNA, and this is probably due to a partial degradation of the RNA molecules during the purification procedure.

In sample 2, the Val-oligonucleotides of about eight and nine residues migrate somewhat faster than those in samples 1 and 3, and this is probably due to the presence of salts in sample 2. In samples 1, 2 and 3 (*in vivo* charging experiments) a spot appears that migrates faster than the Val-pentanucleotide. Although this spot is not produced when total oocyte tRNAs are charged *in vitro* (results not shown) by an *E. coli* extract or by a rat liver extract, one cannot exclude the possibility that it corresponds to a tRNA isoacceptor for valine only charged by the homologous enzyme. This supplementary spot was shown not to be due to free valine, Val-AMP or Val-adenosine (data not shown).

After injection of *E. coli* tRNA^{Val} into the oocytes (sample 5), an ³H-Val-tredecannucleotide appears which corresponds to the T₁ digest of ³H-Val-tRNA^{Val}. No spot corresponding to the digests of endogenous oocyte ³H-Val-tRNA is observed. This is due to the fact that the same number of counts were deposited in all cases shown in Fig. 1, and that after injection of *E. coli* tRNA^{Val}, a 20-fold stimulation in cold TCA-precipitable counts

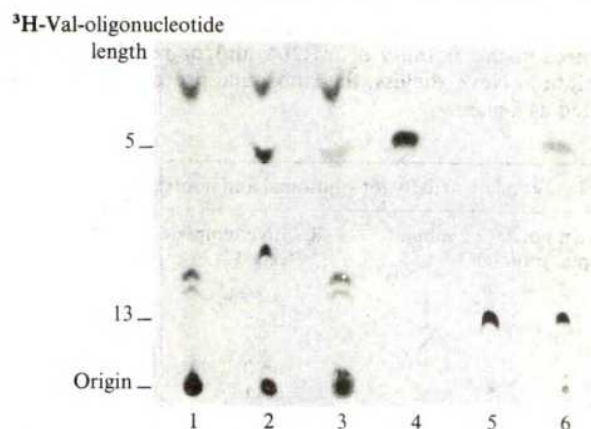


Fig. 1 Separation by TLC on PEI-cellulose plates of the ³H-Val-oligonucleotides obtained after T₁ RNase digestion of *in vivo* or *in vitro* charged (t)RNAs. The *in vitro* aminoacylation with ³H-valine (31 Ci mmol⁻¹; CEA) of unfractionated *E. coli* tRNAs or of total TYMV RNA was carried out as described by Yot *et al.*² using an *E. coli* extract devoid of tRNAs. ³H-Val-(t)RNAs (20,000 c.p.m.) recovered after *in vivo* (see Table 1) or *in vitro* aminoacylation were incubated for 45 min at 37°C in a mixture (15 µl) containing 20 mM sodium citrate, pH 5, 1 mM EDTA, and 0.25 units of T₁ RNase (Sankyo) per µg of Val-(t)RNA. The PEI-cellulose W.R. plates (20 × 20 cm, plastic-backed, with luminescer, Schleicher and Schüll) were soaked in methanol, dried and the digests applied. The plates were developed at room temperature in a stepwise fashion without intermediate drying, using a slight modification of the method of Gupta and Randerath¹⁸; with water to 1.4 cm above the origin; 0.05 M Tris-HCl, pH 6.8, to 4.2 cm; 0.15 M Tris-HCl-8.5 M urea to 7.7 cm; 0.3 M Tris-HCl-8.5 M urea to 10.5 cm; 0.5 M Tris-HCl-8.5 M urea to 14 cm, and finally, with 0.75 M Tris-HCl-8.5 M urea to 16.8 cm. The sheet was dried, fluorographed^{19,20}, and exposed at -70°C using Kodak X-Omat R films. The T₁ digests correspond to the ³H-Val-oligonucleotides obtained from: 1, uninjected oocytes; 2, oocytes injected with total TYMV RNA; 3, oocytes injected with TYMV coat protein mRNA; 4, *in vitro* charged TYMV RNA; 5, oocytes injected with *E. coli* tRNA^{Val}; 6, *in vitro* charged *E. coli* tRNAs.

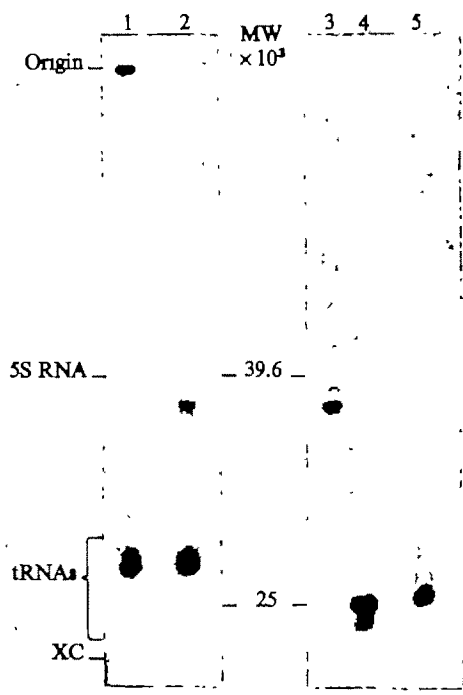


Fig. 2 Analysis by 10% polyacrylamide-7 M urea gel electrophoresis of the TYMV ^3H -Val-RNA charged *in vitro*. Polyacrylamide gel electrophoresis was a modification of the method of Donis-Keller *et al.*²¹: the sample buffer, polyacrylamide gel and electrophoresis buffer were the same but at pH 7, and the Val-(t)RNA samples (10,000 c.p.m.) were not heated before the run. Electrophoresis was carried out at 4°C and 200 V, and the electrophoresis buffer was constantly recycled. The gel was soaked in 10% cold TCA for 1 h, fluorographed^{20,22} and exposed at -70°C. Marker RNAs were run in parallel and stained with ethidium bromide. 1, Uninjected oocytes; 2, oocytes injected with total TYMV RNA; 3, *in vitro* charged TYMV RNA; 4, oocytes injected with *E. coli* tRNA^{Val}; 5, *in vitro* charged *E. coli* tRNAs. XC, xylene cyanol FF.

was observed (Table 1) as compared to uninjected oocytes. Consequently, the corresponding products from endogenous oocyte tRNAs are not visible.

The T₁ digests of the samples presented in Fig. 1 were also separated by paper electrophoresis in 0.5 M formic acid; the conclusions reached agree with those of Fig. 1, namely, that TYMV RNA is aminoacylated when injected into the oocytes.

TYMV RNA as extracted from the virions terminates at the 3' end by -CC. Thus, a positive response to valine aminoacylation indicates that the RNA is recognised *in vivo* by the oocyte tRNA nucleotidyltransferase as well as by the valyl-tRNA synthetase. Solari *et al.*⁹ reached similar conclusions when testing the aminoacylation of injected tRNA^{Phe} devoid of its 3'-terminal adenosine.

It was important to determine the size of the TYMV Val-RNA charged in the oocytes. To this end, ^3H -Val-(t)RNAs charged *in vivo* (oocytes) or *in vitro* (*E. coli* extract) were analysed by electrophoresis on a 10% polyacrylamide, 7 M urea gel (Fig. 2). Total TYMV RNA charged *in vitro* (slot 1) shows in addition to endogenous Val-tRNAs (slot 1) a Val-RNA band migrating between 4S and 5S markers and corresponding to the tRNA-like fragment of TYMV. In the case of TYMV RNA charged *in vitro* (slot 3), an intense band migrating in the same position appears as well as a series of fainter bands of higher molecular weight. Experiments of *in vitro* aminoacylation of TYMV RNA indicate that, with prolonged incubation, these bands of higher molecular weight give rise to the tRNA-like fragment (S. J. and A.L.H., unpublished results).

The *E. coli* system is known to contain RNase P, a maturation enzyme of tRNAs (ref. 10). Extracts containing this enzyme

have been shown

to split TYMV (Val)-RNA, releasing a 4-5S fragment to the 3' end of the genome (see also Fig. 2, similar enzyme(s) experiments here strongly suggest that (a)

As a further control, the oocyte. 4); a main band corresponding to Val-tRNA was obtained, and similarly by *in vitro* charged *E. coli* tRNAs (slot 5).

The results presented here on the structure of a viral RNA is to demonstrate that the tRNA-like structure of a viral RNA is 'processed' *in vivo*, liberating its tRNA-like structure.

Even though these results were obtained using an animal cell, it is likely that similar conclusions would be reached with a plant system. As plant cells are not yet accessible with a plant oocyte might constitute a useful material for such studies, the physiological role of tRNA-like structures in the study of the first place, it is tempting to speculate that the 4-5S RNA fragment formed *in vivo* plays a part in the infected cell by inhibiting, for example, a factor involved in protein synthesis. It is not unlikely that in the infected plant a part of the viral genome could be sacrificed for the formation of such an inhibitor. Indeed, preliminary results (S.J. and A.L.H., unpublished observations) with plant extracts indicate that they also contain (an) enzyme system(s) capable of liberating the tRNA-like structure from TYMV RNA.

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Relationship of proteable surface area thermostability to a

THE pH of an unbuffered protein solution subjected to a continuous temperature increase usually exhibits a single inflection point associated with the cooperative thermal denaturation of the native conformation. Bull and Breese² have used this procedure to determine the midpoints (T_m) for 14 proteins and have found that this parameter is the principal determinant of protein thermostability. We report here our study of 30 additional proteins, selected because of their homogeneous form. With this expanded data base, solvent-accessible surface area was found to result in a better correlation with protein thermostability.

The apparatus used is similar to that described by Bull and Breese², a Radiometer model 25 pH meter, a Radiometer GK 240C combination electrode, a model 425C (Yellow Springs) bridge and thermistor probe, and an Omniscribe dual pen recorder (Houston). Individual protein solutions of about 2 mg ml⁻¹ concentration were dialysed against 0.2 M KCl before use. The protein solution, pH electrode, thermistor probe and a small magnetic stirring bar were inserted into a jacketed vessel surrounded by a grounded wire screen. The temperature of the protein solution was continuously varied by a circulating water bath programmed to increase by 0.83°C per min. Observed pH values were corrected for temperature as described by Goodno *et al.*³.

The T_m values for 30 proteins are listed in Table 1 in increasing value. Each protein preparation used exhibited a single

Coomassie blue staining component when examined by polyacrylamide gel electrophoresis in the presence of detergent, and a single $\Delta pH/\Delta T$ discontinuity over the temperature range 30–90°C. The single exception to this is the preparation of the S1 fragment of myosin, which contains a major component of molecular weight 9.3×10^5 . Scanning calorimetric measurements indicate that this component has a T_m of 43°C. The T_m values listed are the highest values measured in the pH range 4–9. Those listed in Table 1 for nine proteins (6,7,21,22–25,27,30) of the 10 proteins studied in common with Bull and Breese have T_m values agreeing with those previously reported indicating that our execution of the $\Delta pH/\Delta T$ procedure is appropriate. However, our measurements do not support the correlation between T_m and average residue volume (V_R) reported by Bull and Breese². In contrast to their correlation coefficient of +0.96, we find a value of -0.02. Similarly, in contrast to their correlation coefficient between T_m and hydrophobic index of +0.62, we find a value of -0.01. We conclude that the previously reported strongly positive correlation coefficients resulted from examination of a limited number of proteins and do not necessarily reflect any fundamental principle of protein stability.

In searching for an alternative explanation for the observed variation in protein thermostability, we noted that T_m exhibited an imperfect inverse trend with polypeptide chain length. As the ratio of surface to volume for globular proteins decreases with chain length, we have related the observed T_m values to the fractional area of the polypeptide accessible to the solvent. The total surface area of a denatured polypeptide chain, A_t , was calculated from the relationship⁴ $A_t = 1.44M$, where M is the molecular weight of the protein; the solvent-accessible surface area of an individual protein globular domain was calculated using the relationship⁵ $A_s = 11.116M^{2/3}$, and the accessible surface area of a globular domain in a dimeric or square planar tetrameric polymer of identical globular domains was deter-

Table 1 Protein parameters

Code	Protein	Source	pH	T_m	V_r	Φ	$\log M_s$	n
1	Myosin S1 fragment	Rabbit muscle	6.83	43 ± 1	87.6	788	4.97	2
2	Phosphorylase	Rabbit muscle	8.06	48 ± 2	88.9	886	4.97	4
3	Cytochrome c peroxidase	Yeast	6.49	50 ± 1	87.1	831	4.53	1
4	Fumarase	Porcine heart	6.91	53 ± 1	87.1	839	4.68	4
5	β -galactosidase	<i>Escherichia coli</i>	7.48	54 ± 1	86.1	826	5.13	4
6	Trypsinogen	Bovine pancreas	5.80	55 ± 3	79.7	770	4.39	1
7	Chymotrypsinogen	Bovine pancreas	4.90	56 ± 1	80.2	792	4.41	1
8	Lactate dehydrogenase	Rabbit muscle	6.86	56 ± 1	85.7	908	4.56	4
9	Phosphoglycerate kinase	Yeast	7.01	56 ± 3	84.4	832	4.69	1
10	Elastase	Porcine pancreas	6.70	57 ± 1	82.2	799	4.41	1
11	Carbonic anhydrase	Bovine erythrocyte	6.59	57 ± 2	86.2	829	4.47	1
12	Aldolase	Rabbit muscle	6.51	58 ± 1	84.3	792	4.60	4
13	Triosephosphate isomerase	Rabbit muscle	7.39	61 ± 1	82.3	737	4.43	2
14	Catalase	Bovine liver	6.92	61 ± 2	86.3	805	4.73	4
15	Pyruvate kinase	Rabbit muscle	6.97	62 ± 1	84.5	793	4.76	4
16	Adenylate kinase	Rabbit muscle	6.69	62 ± 2	86.0	793	4.33	1
17	Carboxypeptidase	Bovine pancreas	6.69	63 ± 1	86.1	657	4.53	1
18	Alcohol dehydrogenase	Horse liver	7.12	64 ± 2	82.7	830	4.61	4
19	Subtilisin	<i>Bacillus subtilis</i>	6.22	65 ± 2	76.9	739	4.44	1
20	Serum albumin	Bovine plasma	6.40	65 ± 3	86.9	838	4.82	1
21	Haemoglobin	Bovine erythrocyte	6.10	67 ± 1	84.3	873	4.20	4
22	Concanavalin A	Jack bean	6.56	71 ± 2	82.8	846	4.41	4
23	Lysozyme	Hens egg	6.50	72 ± 1	83.0	706	4.16	1
24	Myoglobin	Whale muscle	6.40	76 ± 2	87.8	850	4.25	1
25	Ovalbumin	Hen's egg	6.47	76 ± 2	86.1	866	4.64	1
26	Trypsin inhibitor	Soy bean	7.61	77 ± 1	85.2	878	4.05	1
27	Lactalbumin	Bovine milk	4.50	82 ± 3	88.1	939	4.15	1
28	β -lactoglobulin	Bovine milk	6.10	83 ± 1	88.2	869	4.27	2
29	Superoxide dismutase	Bovine erythrocyte	7.15	83 ± 1	79.0	675	4.20	2
30	Cytochrome c	Horse heart	7.80	83 ± 2	87.0	742	4.10	1

Columns headed by the terms or symbols, pH, T_m , V_r , Φ , $\log M_s$ and n refer to the pH at which the T_m was observed, the T_m measured by the $\Delta pH/\Delta T$ procedure, the average residue volume, the hydrophobic index, the log of the polypeptide molecular weight and the number of polypeptide chains present in the native protein, respectively. The hydrophobic index was calculated as described by Bigelow¹¹.

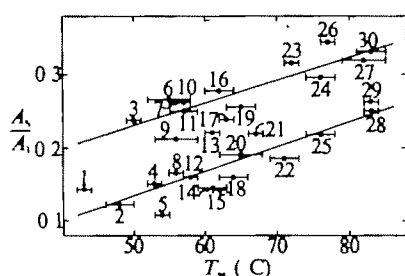


Fig. 1 Relationship of fractional polypeptide area accessible to solvent, A_s/A_t , and protein thermostability, T_m . A_s is the polypeptide surface area in $\text{\AA}^2$ accessible to the solvent in a native globular protein, A_t the total accessible surface area of a denatured protein, and T_m the midpoint of the thermal denaturation detected by the $\Delta pH/\Delta T$ procedure. The open circles indicate monomeric proteins and the filled circles polymeric proteins. The number associated with each experimental value indicates the identity of the protein as encoded in Table 1. The lines represent least-squares analyses of the values for the monomeric (except for proteins 20 and 25) and the polymeric proteins.

mined using the factors⁵ 0.857 and 0.714, respectively, to account for the surface lost in the monomeric unit by polymerisation. As seen in Fig. 1, the values for the 30 proteins seem to cluster about two linear relationships with a common slope, one relationship being populated exclusively by monomeric proteins and the second principally by polymeric proteins.

The slopes of these lines suggest that increasing the internal area of these proteins at the expense of surface area decreases the thermostability in the temperature range considered. As hydrophobic index and percentage apolar atoms are independent of polypeptide chain length, and as polar atoms are preferentially located on protein surfaces, it follows that more polar atoms, particularly those of the peptide backbone, must be internalised as the fractional surface area is diminished. Such internalisation of polar atoms in contrast to apolar atoms presents a problem, for burial of a polar atom not complementarily hydrogen bonded in the internal apolar environment is energetically unfavourable. We propose that as more polar atoms need to be internalised to fold longer polypeptide chains into globular units, the probability of noncomplementary burial increases, leading to the decreased thermostability indicated in Fig. 1. In the case of burial of peptide backbone polar atoms, it should be noted that the amount of secondary structure, a common method to pair these atoms complementarily, is independent of chain size. The relationship exhibited by the monomeric proteins predicts that a polypeptide with a molecular weight $>60,000$ folded into a single globular domain would be unstable when present in an organism maintained at 37°C . Fisher⁶ has suggested that large polypeptide chains would be stabilised by forming asymmetrical structures, thus increasing their surface to volume ratios.

Alternatively, the results shown in Fig. 1 suggest two alternative ways in which the thermostability of larger polypeptide chains can be enhanced. One involves using globular surfaces to contribute to conformational stability through association of globular subunits to form polymers. Analysis of subunit interfaces suggests that polar complementarity provides the selectivity while apolar interactions provide the added stability for these inter-subunit interactions. The relationship exhibited by the polymeric proteins in Fig. 1 suggests that polypeptide chains with molecular weights up to about 160,000 would be stable at 37°C provided that such chains fold into globular units and that the globular units polymerise. This limitation would encompass the polypeptide chain lengths of all globular polymeric proteins⁸. The results shown in Fig. 1 also suggest a second way to increase the thermostability of larger polypeptide chains, namely, folding a single chain into multiple globular domains. A case in point is the apparent enhanced thermostability of phosphoglycerate kinase, protein 9. The crystallographic structure⁹

of this protein demonstrates that its single polypeptide chain folds into two globular domains of nearly equal size, with minimal interdomain contact; this conformation would increase the fractional surface area relative to folding into a single globular domain and accordingly increase thermostability. Analysis¹⁰ of the sequence of serum albumin suggests that this protein folds into three globular domains of closely related sequence, probably the result of fusion of duplicated structural genes. The position of the T_m for serum albumin, protein 20 in Fig. 1, suggests that its structure resembles that of a covalently linked polymeric protein. The position of the T_m for ovalbumin, protein 25, in Fig. 1, suggests that its relatively large polypeptide chain is also folded into more than one globular domain. Unfortunately, no sequence or crystallographic information is yet available for ovalbumin to validate this suggestion.

In presenting our analysis of thermostability in terms of the size of globular domains and polymerisation of domains, we recognise that other considerations such as the presence of disulphide bonds, ligation of strongly bound metal ions, haem moieties and other cofactors, packing densities, and intra-surface interactions will also influence thermostability. Indeed, such considerations may be partly responsible for the scatter of the values about the linear relationships considered in Fig. 1. We also recognise that the inverse A_s/A_t dependence on T_m for globular proteins produces an absurd extrapolation, namely, that the most thermostable protein would have no buried area. Clearly, other considerations must limit this relationship. However, it seems to extend to include pancreatic trypsin inhibitor, which has a A_s/A_t value corresponding to a T_m of 111°C , as no $\Delta pH/\Delta T$ discontinuity could be detected for this protein at temperatures up to 98°C . Although, as more information becomes available, our analysis of thermostability may suffer the same fate as the average residue volume correlation, a cursory review of thermal inactivation measurements for a variety of enzymes suggests that size of globular domains and polymerisation are critical to protein thermostability.

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Errata

In the letter 'The units of calcium conduction in *Helix* neurones' by N. Akaike *et al.*, *Nature* **274**, 379-382, ref. 6 should read: Kostyuk, P. G., Krishtal, O. A. & Pidoplichko, V. I. *Nature* **257**, 691-693 (1975). (This replaces the reference to a 1977 paper by the same authors.)

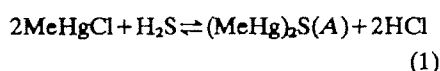
In the letter 'Single crystal analysis of the structure of stishovite' by W. Sinclair and A. E. Ringwood, *Nature* **272**, 714, in paragraph 4 line 2 the space group should read: $P4_2/m2_1/n2/m$.

In the letter 'Origin of pregalactic microwave background' by M. J. Rees, *Nature* **275**, 35-37, in paragraph 1 lines 5, 6 should read: $(\delta\rho/\rho) \propto M^{-\alpha}$ ($\frac{1}{2} \leq \alpha \leq \frac{2}{3}$) (refs 12-15). Also, in paragraph 6 lines 1, 2 should read: Nucleosynthesis could have supplied heavy elements by $t \approx 10^7$ yr.

matters arising

Volatilisation of methylmercuric chloride by hydrogen sulphide

TREATMENT of methylmercuric chloride with hydrogen sulphide in aqueous solution has been shown by Rowland *et al.* to result in volatilisation of mercury¹. The volatile product was not identified but was considered to be a 'volatile sulphur compound of methylmercury'. Volatilisation of mercury and concurrent lowering of the pH of the aqueous solution¹ can be explained by formation of bis(methylmercuric) sulphide (A).



This reaction has previously been carried out in ethanol^{2,3}. The foul odour of A (ref. 2) indicates that it is volatile, even though it is a solid at room temperature. Loss of mercury caused by ammonium sulphide¹ can also be attributed to formation of A.

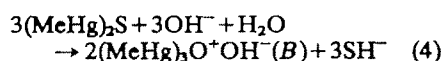


The reduced effectiveness of this reagent compared with hydrogen sulphide¹ can be explained by the simultaneous occurrence of reaction (3).

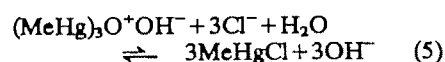


Reactions analogous to (2) and (3) have been observed between MeHgBr and Na₂S (refs 4, 5).

Absorption of the volatile mercurial by alkali¹ can be attributed to formation of the known⁶ *tris*(methylmercuri)oxonium hydroxide (B) (contrast with doubtful existence of MeHgOH⁶).



The ionic character of B would explain the failure of the base-soluble species to undergo chromatography¹. Although chloride ions, from absorption of volatilised HCl, would also be present, the equilibrium (5) would lie well to the left. However, extraction with toluene would remove the non-ionic methylmercuric chloride and drive the equilibrium to the right, explaining recovery¹ of MeHgCl by this method.



The volatilisation of methylmercury could also be explained by formation (6)



of MeHgSH (reported preparations^{7,8}), and reactions (2) and (4) could be modified to accommodate this. However, reaction (6) has not previously been demonstrated, whereas there is precedent for (1)–(3)^{2–5}, hence A is preferred as the source of volatility. Since A is unstable, decomposing by (7)², dimethylmercury and mercuric sulphide would probably be the ultimate products from MeHgCl and H₂S in an environmental situation.



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World's oldest animal traces

BIOLOGISTS and biogeologists would like to know when multicellular animal life began. Well authenticated records show that it was present by ~680 Myr ago, but older records have not withstood scrutiny. Clemmey's report¹ of metazoan burrows from 1,000 Myr old rocks in Zambia therefore excited great interest. I have examined the specimens and seen Clemmey's coloured slides of the outcrops, and this examination and subsequent research led to a different conclusion from his. The Zambian burrows are undoubtedly metazoan and indigenous to the sedimentary deposits in which found, but they are of post-depositional origin and related to a geologically recent surface. Evidence for this conclusion rests in the bleached, saprolitic nature of the host rock and the contrasting red and pink banded burrow-fillings, containing rounded, ferromanganese-coated quartz grains that are much larger than particles of the adjacent matrix. Such characteristics suggest introduction of burrow-filling material from a relatively recent lateritic weathering surface.

Further study and inquiry among students of the social insects led to the additional conclusion that Clemmey's burrows are the work of termites, digging through and parallel to the softer layers of the steeply dipping saprolitic rock to obtain water for humidification of their nests. From publications and correspondence with termite specialists M. Lüscher, Ch. Noirot, M. L. Roonwal and W. A. Sands, I found that termites do burrow to depths of 70 or more metres to reach the water table, that they burrow preferentially in the least resistant parts of the rock, and that they backfill unused burrows with surface matter producing laminated burrow-walls and a banded 'spreite' pattern like that illustrated by Clemmey. His observation that the burrows are parallel or nearly so to the bedding, and that they favour particular stratigraphic levels, would be consistent with descent along the softer of the steeply dipping strata of the Zambian Copperbelt.

The above comments apply mainly to burrows of the type of Clemmey's Fig. 1. The oval profile, pinching and swelling along their lengths, and terminal branching of such burrows is also termite-like. The bioturbation of his Fig. 2 may represent a zone of termite foraging or collapse. Specimens like his Fig. 4 (with a hollow interior) resemble the covered passageways through which termites cross lighted areas.

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CLEMMY REPLIES—I agree that the burrows I described are only obvious after weathering, but this is a feature shared by much of the internal fabric of rocks and in this case has an explanation. The red and pink banding results from weathering of the microchemical/mineralogical differences between bioturbated and non-bioturbated sediment. Within the burrows it reflects the differences between sediment passed around the body or through the gut of the animal and such differences are to be expected in bioturbated sediment¹.

The burrow, as a microreducing environment, attracted the precipitation of iron sulphides, which ideally would be more concentrated in the zones of the bioturbated sediment richest in organic matter. On weathering the ferrous iron is oxidised to the ferric state and hence the

red and pink shades. The country rock depending on its iron content weathers cream, brown, or pink. Within the bioturbated sediment and sometimes in the country rock are pools of authigenic quartz such as may be formed when silicates are breaking down under biogenic attack or during weathering and are releasing large amounts of silicic acid. These are the 'quartz grains' referred to by Cloud.

If the burrows were made by termites, why did those making type A (ref. 2) burrows live only above the 2 m of strata at the base of the Orebody Member whilst the succeeding 2 m are characterised only by those making type B burrows²? And why is the unit containing the burrows overlain by hundreds of metres of identical lithologies which show no signs of bioturbation. I believe that the burrows are not those of termites nor are they lithologically controlled, but they are paleofacies-controlled. The transition type A to type B burrows reflects the transition from intertidal to lagoonal/lacustrine sedimentation, as indicated by facies studies³.

Total destratification, such as indicated by my Fig. 2 (ref. 2), produces a swirled fabric in the rock; this is explained only by invoking contemporaneous sedimentation and bioturbation. For while small tunnels of termite origin are at least conceivable, complete fluid mixing over a thickness of several centimetres is not. This fabric implies that the medium undergoing bioturbation was granular and wet.

The idea that type B burrows represent covered passageways is not feasible. The burrows are an integral part of the rock, are not confined to exposed bedding surfaces and can be revealed by excavating the strata. They have pellet-formed walls which may show disintegration at the burrow termination into equidimensional pellets. Again these are part of the rock and not surficial features.

As far as it is possible to tell, the burrows (unequivocally metazoan according to Cloud) were formed contemporaneously with the sedimentation of the containing units about 1,000 Myr ago.

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No paradox in the control of energy intake

AS Rothwell and Stock¹ note their results refute the notion that a particular size of energy store precisely controls energy

intake in rats. Other reports²⁻⁴ agree with this. However, contrary to what is claimed, their results do not necessarily reveal a paradox or physiological enigma in the control of energy intake in the rat. They are clearly compatible with the theories of Kleiber⁵, Adolph⁶, Ugolev⁷ and Booth⁸, in which energy metabolism controls feeding and the energy store is only one component in the feedback system. Rothwell and Stock state that our model⁹ has yet to include effects of body energy increments. On the contrary, in a publication which they cite¹⁰, and elsewhere¹¹, we have included a representation of effects of energy store size on intake through energy metabolism. We also emphasised that although the energy store may settle at a particular size¹¹⁻¹³, there is no privileged size either achieved or used as a homeostatic set-point. In these simulations the precision of defence and (if a specific size is maintained) the value defended depends on a compromise between the small but persistent graded effect of store size on metabolism and all the other dietary and endocrine conditions affecting metabolism.

Rothwell and Stock also state that it is difficult to conceive how such a model could explain an increase in fat deposition rather than a decrease in intake when energy supply is in excess of requirements. However, our model probably provides several ways of increasing efficiency without altering intake. For example, a change in regimen might divert a higher proportion of absorbed energy into fat, while also reducing the faecal excretion of energy or the thermic effect of feeding. All these effects would be liable to increase the size of the energy store in the model. The extra metabolism from the increased retention of energy intake would help reduce intake, and the extra diversion after absorption would help to increase it.

The importance of Rothwell and Stock's data lies in the questions raised as to the exact mechanism(s) by which dietary changes can alter the relations among the processes of utilisation, storage and intake. Quantitative simulation is of similar importance.

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ROTHWELL AND STOCK REPLY—We apologise to Booth and Toates if we have neglected those aspects of their feeding model that take into account energy storage. Nevertheless, we were well aware (and said so) that the increased fat deposition of our animals results from an increased metabolic efficiency (almost certainly due to a decreased thermic effect of feeding), thus providing a larger fraction of total ingested energy available to metabolism. According to Booth and Toates, this excess energy would either reduce total energy intake or, if diverted to fat storage, help to increase intake. In fact, we found that total energy intake remained at precisely the same level as that in the free-feeding control animals.

We would also suggest that an energostatic approach fails to account for the differences between the two types of energy load used in our experiments, that is, fat and Complan. If one accepts Booth's proposal¹ that it is the availability of energy that determines intake, it is logical to assume that isoenergetic stomach loads will have similar effects on voluntary energy intake. In our experiments, however, rats tube-fed 34% of normal intake precisely compensate for the load when it is a balanced nutrient mixture, but over-eat by 15-16% when the load is comprised of fat. This suggests that an energostatic approach may be too simplistic and, since our original letter was published, Savory has written to us supporting this view. He obtained very similar results to ours by feeding quail with diets of varying energy density², and one of his conclusions from this work was that "... differences in energy expenditure were not balanced by differences in nutrient intake, so perhaps the quail were regulating food intake to meet their requirements for some dietary component other than energy".

There are many attractive aspects of the energostatic model of Booth and Toates, and we regret that they did not take advantage of their computer simulation to substantiate the claim that our results are compatible with their feeding model. We hope that they will do so and if necessary, as we suspect it will be, make appropriate alterations.

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Induced suction and lithospheric plate pull

SHOEMAKER¹ proposes to explain the driving force of plate tectonics as 'induced suction' by the low density sediments in the trench. However, his calculation, which is based solely on fluid statics, does not tell us what the energy source for the motion is, neither does it tell us anything essentially new about subduction dynamics.

To illustrate this, consider an experiment in which a high viscosity fluid is flowing steadily over the edge of a horizontal table. The fluid which has just passed over the edge responds to the unbalanced vertical force of gravity, and this force is 'communicated' to the rest of the fluid by means of a horizontal pressure gradient (that is, the upper surface of the fluid is no longer horizontal). A statics calculation of the type made by Shoemaker would demonstrate that the table edge provides 'suction', but this obscures the fact that the cause of the motion is the vertical force of gravity. The essentials of the argument still apply if the rheology is more complicated.

In the plate tectonics context, the value Shoemaker obtains for the 'suction' is nothing more than the downward force on the subducting slab, presumably caused by gravity². Trench depression can be predicted by a dynamical calculation³, and the accumulation of sediments at the trench is an effect of plate motion, not a cause.

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SHOEMAKER REPLIES—Induced suction¹ is a consequence of the combination of two conditions being satisfied by the subduction process: first, that the fluids or materials above and below the subducting plate have different densities and second, that the subducting plate bends beneath the overriding plate in contrast to the idealised situation where it passes from the horizontal to the downward dipping state through a sharp corner². The force of induced suction is not the downward force of the subducting slab nor is there any direct connection between these two forces. There is an obvious indirect connection in that the downward force of the subducting slab has a major influence in creating the downward bend which causes the induced suction.

The major energy source for plate motion may be downward pull of the subducting slab; it might be something else. However, any consideration of

energy is immaterial to the argument presented for 'deriving' the existence of induced suction. The argument was based on equilibrium which has equal validity with energy principles.

There is no connection between the example of a highly viscous fluid flowing over the edge of a table and induced suction. The 'communication' between the downward flowing fluid and the horizontal flowing fluid on the table is provided by the table edge; the horizontal component of the force exerted by the table edge upon the fluid pulls the fluid along the table. This is not induced suction. Moreover, the fluid will certainly flow if, for example, there is air above and below the fluid 'slab'.

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Circadian variation in immune response to oxazolone

FACHET AND ANDO¹ suggest that cellular and humoral responses to oxazolone are under the control of *H-2* genes. Their evidence for the control of cellular immunity was based on the differences between inbred and *H-2* congenic strains of mice in the magnitude of the delayed hypersensitivity response to oxazolone. This was measured in the ears of sensitised mice as the difference in ear thickness measured immediately before and 24 h after challenge. Using this method, we have demonstrated in rats that the magnitude of the maximum immune response depends on the time of day at which oxazolone is applied to the ears². Subsequently, we have shown that there are circadian variations in the skin response to the injection of purified protein derivative in man³. It seems that the time of day at which the antigen is encountered influences the magnitude of the delayed hypersensitivity response.

Most investigators carry out experiments at the same time of the day. We would welcome an assurance from Fachet and Ando on this point, for if their experiments were not standardised to one time of challenge for all the mouse strains, this affects the interpretation of their findings. Additionally, if the oxazolone challenges were given over a long time span within a single day this might affect the results adversely, as the maximum observed response to challenge with oxazolone at 10.00 in the rat is over eight times the minimum response at 16.00.

Data on the time of challenge and the lighting regimen of the animals are not available in the paper from the Hungarian Unit which therefore does not contain sufficient details for the experiments to be repeated, surely a prerequisite of any scientific publication.

Biological rhythms influence many cell-mediated and humoral immune processes with effects which are often of a high order of magnitude. Our recent finding (unpublished) that the delayed hypersensitivity response to oxazolone can be manipulated by altering the lighting regimen of the animals is a further demonstration that the basic principles of chronobiological experimentation need to be introduced immediately into immunological studies.

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FACHET AND ANDO REPLY—The points raised by Pownall *et al.* are of interest and we make the following comments. All experimental mice of the *H-2* congenic strains used were sensitised and their delayed-type hypersensitivity (DTH) to oxazolone was tested between 09.00 and 11.00. The same timing of experimental procedures was applied when the DTH to oxazolone was tested in a larger number of animals of the same strain and in mice of other inbred mouse strains. The same conclusion was reached concerning the effect of genes within and outside the *H-2* complex. A single strain has not been independently tested; a series of different strains only has been investigated and used for comparison¹.

Within the scope of our experiments we found no diurnal variation in responsiveness on testing *H-2* congenic mouse strains with oxazolone but it well might occur in different conditions.

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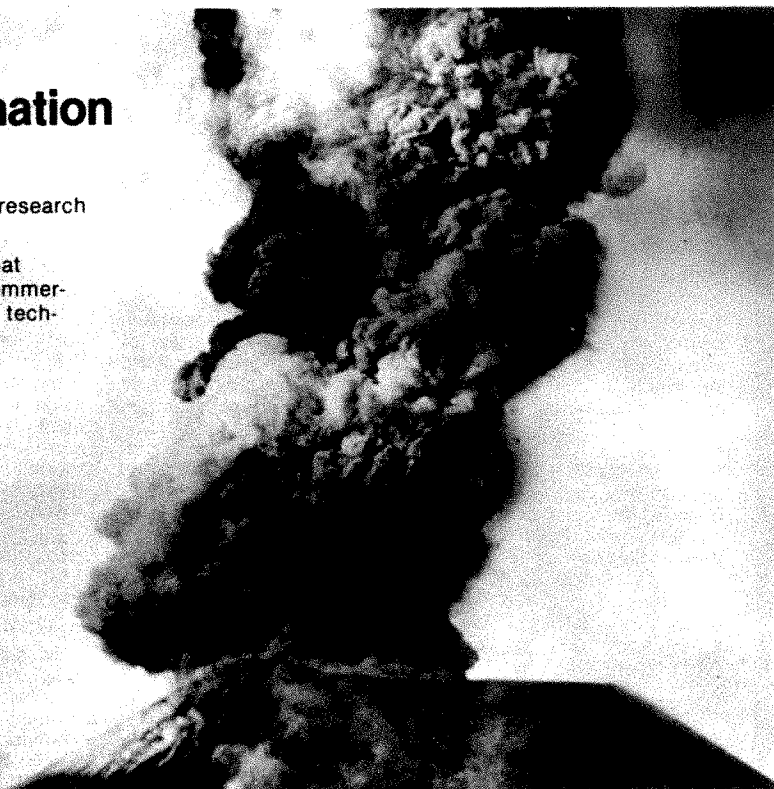
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edited by **C.A. Croxton**, *Department of Mathematics, University of Newcastle, New South Wales, Australia.*

The most recent major reviews have outlined the essentially sound statistical mechanical foundation of the physics of simple liquids as it stood in the mid-sixties. Since then, significant advances have been made into more difficult and controversial fields, in particular quantal and critical systems, irreversibility, transport phenomena, phase transitions, molecular and polymeric systems, liquid crystals and the role of triple effects. These and other topics are discussed in this collection of review papers. The volume not only attempts to consolidate existing knowledge and understanding but also focuses attention on some of the major outstanding concepts and difficulties which remain unresolved. The book also indicates the development of the subject of liquid physics away from simple fluid systems towards new applications in adjacent fields.

March 1978
0471 99445 6

602 pages
\$55.00/£25.00

Introduction to Liquid State Physics

by **C.A. Croxton**, *Jesus College, Cambridge.*

This book was written in the belief that the theory of liquids is now ready for dissemination on to a wider and more general audience — to chemists, metallurgists, biophysicists and engineers, not to mention the non-specialist physicist who also needs to know something of the liquid state. Short accounts are presented of irreversibility, phase transitions and transport phenomena, liquid metals and liquid-crystal systems. Inevitably, some selection has to be made, and therefore the more complex molecular systems such as glasses, water and liquid mixtures could not be included.

The equilibrium theory of simple fluids is dealt with at length, together with the statistical thermodynamics of the free liquid surface, and extensive illustrative use is made throughout of the computer simulation schemes which are now central aids in the theoretical development of the subject.

April 1975
0471 18933 2
0471 18934 0

296 pages
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Introductory Eigenphysics

An Approach to the Theory of Fields

by **C.A. Croxton**, *Jesus College, Cambridge.*

This book gives a physical introduction to the mathematical treatment of a large number of problems taken from physics, geophysics, astronomy and chemistry. Emphasis throughout the book is on the physical basis of the problems discussed, the mathematical background is relegated to a series of appendices so as not to interfere with the physical development. Unlike other treatments which suffer from a surfeit of mathematical rigour at the expense of physical understanding, in this approach the mathematics is regarded firmly as a tool of the physics. It is no coincidence that so few differential equations describe so much physics, and in the book the author fully exploits this unifying basis of the subject in an attempt to break down the conventional division between quantum and classical mechanics, between physical optics and electron scattering, or between electrostatics and aerodynamics. Supplementary problems are given at the end of the chapters.

"There are many interesting exercises for the reader... I do, however, warmly recommend the book to teachers."

Michael Berry, *NATURE*

November 1974
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286 pages
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Introduction to Liquid State Chemistry

by **Y. Marcus**, *Department of Inorganic and Analytical Chemistry, The Hebrew University, Jerusalem.*

This introduction to liquid state chemistry applies the tools of statistical mechanics and thermodynamics to the equilibrium properties of liquids and liquid mixtures, so that reactions and interactions phenomenologically described can also be understood, and made the basis for predictions regarding systems not yet studied. Non-polar liquids, electrolyte solutions, molten salts, non-aqueous solvents, and liquid metals and alloys are brought together in one volume and discussed from a unified point of view, based on common models.

June 1977
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376 pages
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Intermolecular Forces

by **T. Kihara**, *Department of Physics, University of Tokyo, Japan*

This is a survey of the theory of intermolecular forces. Topics such as quantum-mechanical perturbation theory, statistical mechanics and the kinetic theory of gases, and structures of molecular crystals are treated assuming only an elementary knowledge of quantum mechanics.

June 1978
0471 99583 5

192 pages
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Advances in Chemical Physics Vol. 37

edited by **I. Prigogine**, *Université Libre, Brussels,* and **S.A. Rice**, *University of Chicago.*

This reports, interprets, and evaluates progress in the field of chemical physics. Topics covered have been chosen by the editors for their timeliness in the field and are treated comprehensively and without limitation of space by their respective authors.

Further volumes are in preparation. (Series: *Advances in Chemical Physics*)

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Organic Chemistry, Revised Printing

by **T.W.G. Solomons**, *University of South Florida.*

This gives a unified introduction to fundamental concepts, functional groups, and major types of reactions. It emphasizes biochemical and biological applications, and, where appropriate, parallels reactions which occur in the organic laboratory with those that occur in living cells. Special topics which augment the fundamental concepts are interspersed throughout the text.

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reviews

Rationality and actions

A Rational Animal, and Other Philosophical Essays on the Nature of Man. By Anthony Flew. Pp. 245. (Clarendon/Oxford University Press: Oxford, 1978.) £5.95.

THE attempt to demonstrate on *a priori* grounds that natural science can never fully explain human behaviour has been a fashionable parlour game amongst philosophers for some time. This question—and associated problems hinging on the nature of freedom of choice and rationality—is addressed by Professor Flew with somewhat more caution and subtlety than has been displayed by many of his predecessors. Each of the essays of which *A Rational Animal* is composed attacks the views of one or more other thinkers: the targets range from Hume to Lenin, and one cannot help feeling that many of them, like Skinner and Freud, were so philosophically naive that they do not require the weight of artillery that Anthony Flew brings to bear. Moreover, his method of proceeding has the serious disadvantage that amidst the smoke of battle, it becomes difficult to discern the position occupied by the author himself. One longs for a clear statement of his own views, and if in what follows I have misinterpreted him, he is at least partly to blame.

He bases much of his argument on our knowledge that we are free to choose: whenever we perform a voluntary action, we know that we could always have acted otherwise had we so chosen. He takes this alleged fact to be irreconcilable with Laplacean determinism which proposes that each state of the Universe is necessarily caused by its antecedent state. The fact that we can make voluntary movements implies that "What there certainly cannot be . . . is an unbroken chain of sufficient physical causes stretching back indefinitely. It would therefore seem that the central nervous system must either be or contain an apparatus of which the total input does not contingently necessitate every element of total output." He acknowledges that this suggestion is made reluctantly and elsewhere he seems to write as though there were no need for this drastic solution, which implies that the matter in our brains

obeys different laws to those currently thought to hold in inanimate matter. As has often been noted, the invocation of Heisenberg's uncertainty principle is of no help as choices are not made at random and therefore cannot themselves be determined by random events at the quantum level.

Flew's own arguments do not in fact seem to necessitate his conclusion. It no more follows from the fact that we feel free to choose that we are free to choose (in the sense that our choice is not determined by the state of our brains) than it follows that someone who is convinced he can grow wings can grow wings. Moreover, to believe that someone might have chosen differently is surely not inconsistent with believing that he would only have done so had he been in a different antecedent psychological state. This is not to deny the difference between voluntary and involuntary acts. It could be argued that we feel free to choose because we have no way of knowing with certainty what we will do until we decide to do it: in order to know in advance, we would have to simulate at time A our state at time B, and the possible effects of undertaking this simulation at time A on our state at time B could not be included in the simulation itself.

Flew concludes that human actions can never be explained and predicted by physiology: he is correct, but for the wrong reasons. We can no more explain human activity in terms of goings-on in individual nerve cells than we can explain how a computer program works in terms of transistors

To understand how any organised system of matter works, we must develop concepts appropriate to that kind of system. We can understand and predict the hunting behaviour of a system incorporating negative feedback only in terms of control theory not in terms of previously existing physical concepts. Similarly, the scientific explanation of behaviour requires the development of concepts appropriate to understanding the organisation of the brain in relation to the external world: such concepts may not be physical or even physiological, but in as far as they are both precise and render human actions intelligible in a rigorous way, they are nonetheless scientific. Nor does the need to evolve such explanatory concepts suggest that behaviour is undetermined any more than the fact that computer programs cannot be understood in terms of transistors suggests that the outcome of running a program is undetermined.

A small beginning has been made towards developing the requisite explanatory conceptual system within the disciplines of cognitive psychology and artificial intelligence, and it is a pity that philosophers who write about rationality and actions do not examine these disciplines rather than castigating psychoanalysts, who are not scientists, and physiologists, who work at the wrong level of abstraction to be in the business of explaining human actions. □

Stuart Sutherland is Professor of Experimental Psychology at the University of Sussex, UK.

Commissure-section studies

The Integrated Mind. By M. S. Gazzaniga and J. E. LeDoux. Pp 168. (Plenum: New York and London, 1978.)

THE rarity of certain kinds of neurological condition is well recognised. Any investigator who obtained access to more than one case of visual or tactile object agnosia or more than one case of cortical blindness or achromatopsia in a lifetime would count himself lucky indeed.

Surgical brain bi-section falls into a related category. Those of us who work outside of the USA will never have the opportunity to examine such patients (except by visiting the USA), because only in America have the fibre pathways connecting the cerebral hemispheres been surgically divided for the relief of epilepsy. Three separate major series (and one smaller series) of commissure-sectioned patients exist. The earliest patients were investigated before modern and subtle tests of brain

—behaviour relationships had been devised. The next (Californian) series is associated with the names of R. W. Sperry and J. Bogen. These patients were carefully studied after exceptionally radical commissure-section; but, regrettably, only a proportion of the patients have been described in the literature. The most recent (New England) series is smaller in number and the patients underwent less extensive neurosurgery.

Michael Gazzaniga, the senior author of this book, has a unique advantage: personal experience of both the Californian and New England series of patients. In addition, he is the originator of many important experiments on 'split-brain' monkeys, and he described some of the earlier findings in 1970, in his book *The Bisected Brain*. In the present book we are brought up to date with recent findings from the New England series. The intention is to "... reestablish a basic, sober framework for considering studies on cerebral commissurotomy", as a counter to "... a barrage of popular and overdramatized accounts ... largely written by people who had never seen a patient" (p6).

Will the reader (if he is not put off by the title of the new book, or by the statement on p1 that "The corpus callosum, the largest fiber tract in the human brain, contains over 200 million neurons ...") find what is promised? Sadly, no. The majority of observations reported in the book have already been published in the journals. There is no systematic attempt to group the

observations by the extent of surgery, by the duration or amount of pre-surgical pathology, or by the length of time since surgery at the time of testing. Indeed, a large part of chapter 3 is based on the performance of a single patient (P. S.). Yet we read (p85): "In contrast to all the other patients examined in both series is case P. S.", in whom "... we observed an incredible range of language skills in both hemispheres ...". Certainly, the observations forming the basis of chapter 3 were non-linguistic, but no reasons are given why they were not confirmed on other more typical patients from either series.

And yet, in another framework, the book can be recommended. It is essentially an account of the authors' wide-ranging views on such topics as the origins of lateral asymmetries, the role of language, the basis of intelligence and consciousness, the relationship between perception, imagery and memory, and many more such issues. Most of the opinions are original and provocative; and as opinions they are well argued. In due course adequate evidence to support these opinions may be forthcoming. Meanwhile, the book will serve as an intellectual stimulus to a wide range of neuroscientists who are accustomed to making only painfully slow progress in their own laboratories or clinics: given an "integrated mind", what limit is there to our theorising?

G. Ettlinger

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of what is, as FOE indeed rightly stress, an international common property resource, is not only unjustified but is dangerous. Not only do Soviet fleets operate under significantly different economic parameters, but, as again the FOE stress elsewhere, the USSR has much to gain from any curtailment of solely private capitalistic exploitation.

Overall, the book is fairly good value as it is full of the sort of data and arguments that anyone interested in whales would want to see. It suffers from being an update of a 1975 version, which was an update of the 1972 original. By now it has had a total of 17 authors, and accordingly the deliberately simple style is now patchy, frequently staccato and occasionally irritating. Presentation of the data is generally satisfactory, although some still refers only to 1974-75 which is perhaps disappointing. The few illustrations are rudimentary, but the bibliography is appropriate for the context. The stated objective of the book is to spread information to a wider public, and in this it is likely to succeed. Those in a position to make influential decisions, however, will want to study elsewhere too.

Michael Dunn

Michael Dunn is Principal Lecturer in the Marine Resources Research Unit at Portsmouth Polytechnic, Portsmouth, UK.

Whale primer

Whale Manual '78. Friends of the Earth. Pp. 152. (FOE: 9 Poland Street, London, 1978.) £2.

WHALES are by now a well established *cause célèbre* of the conservation movement, popular on television, and almost universally admired for their benign dispositions and mournful singing. So it comes as something of a jolt to digest the idea that whales could produce a sustainable 2 million tonnes of protein annually (some say), worth perhaps some \$690 million with present prices and utilisation patterns. This little book may help to provide the information to help develop a balanced view on whales and their management, although it remains an enigmatic subject in a manner in which the management of herrings or anchoveta is never likely to be.

In many ways this book is quite excellent. It is ideal as a brief, fact-filled primer on whales and the man-

agement thereof, as long as one accepts that the authors have a (tenable) point of view which significantly colours the text. Not that the contents exclude other people's views, but they are dealt with somewhat erratically. For example, there is some lack of understanding of the reality of economic life, allied to a consistent undertone that, really and truly, whales ought not to be considered as a resource at all but as some sort of monument to man's humanity to mammals. This lack of appreciation of economic factors is illustrated in chapter 6 where the justification for discussing the economics of the whaling industry solely on the basis of the Japanese industry and not that of the USSR is partly the fault of a deficiency of data but mainly results from the fact that the same economic arguments apply to both nations; and in any case slaughtered whales are not interested in "the nicety of distinction between state capital and private capital" (p46). Although the whales may not be bothered in the short run, to dismiss this vital distinction in the exploitation

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obituary

D. R. Chick

PROFESSOR D. R. CHICK, F.I.E.E., F.Inst.P., F.I.E.R.E., Head of the Department of Electronic and Electrical Engineering at the University of Surrey since 1965, died suddenly at his home in Surrey on 11 June 1978 aged 61.

He was one of the many young scientists in the 1930s who began their working life on the applications of radar, which were so vital to the winning of the war. His experiences in the field in wartime conditions, where it was so essential to impress the young local commanders that the civil scientists were not wasting everybody's time, surely helped him form some of his strongest characteristics: a quick and accurate assessment of people, straight, often blunt speaking, a sound scientific and economic judgment, and not least a strong personal loyalty to his own staff and colleagues. One such experience where the accommodation offered to him and his co-workers fell far below that expected considering their equivalent army rank led to a walk-out, much to the distress of the commanding officer, who to save his own reputation swallowed his pride and begged their return.

Immediately after the war, Chick turned to industrial research, joining the A E I company at the new research laboratory of Aldermaston Court. For 17 years, he was in charge of various nuclear physics activities of the company and made large contributions in several different ways. His group developed and constructed electrostatic generators and a helical particle accelerator for basic physics research, and among much fundamental work he and colleagues measured excited levels in arsenic 75 produced by radiative capture of protons by germanium 74. This led to a patent application in 1957 relating to the production of p-n junctions in germanium or silicon by nuclear transmutation following irradiation with protons. It is significant that today transmutation doping of silicon by neutrons is used for special devices.

Also, with Sir George Thomson, a large section was devoted to the thermonuclear research programme, so important in future fusion reactors, using a torus named Sceptre. In 1957, this group, working closely with a Harwell team possessing a larger torus

named Zeta, announced the production of neutrons from the discharge and subsequently studied some of the instabilities which develop in toroidal gas discharges carrying large currents.

His third major achievement in this period was the installation of a 5 MW pool type research reactor designed and developed by his group in conjunction with A E I John Thompson Ltd. This reactor was the first publicly owned one and received reactor licence no. 1 in the U.K.; it was officially opened by H.R.H. the Duke of Edinburgh in 1959. It was used for research and contract work and two similar reactors were sold by A E I John Thompson, one in Germany and one to the Atomic Energy Authority at Aldermaston.

In the mid-60s, it was appropriate that this wealth of experience in both the civil service and industry should be passed to one of the new universities which resulted from the publication of the Robbins report. When Professor Chick joined the then Battersea College of Advanced Technology as Head of the Department of Electrical and Control Engineering, he had a decided policy that his department would become known for both its research and industrial liaison as well as for its academic achievements.

He grouped his research effort into a few main projects, and his forward thinking is demonstrated by the fact that in 1966 he formed a team to work on ion implantation, then a speculative research area, now a major part of microelectronics technology, and he saw this group grow and become recognised as one of the major ones in the U.K. with a good reputation abroad.

He also formed an industrial electronics group which made the expertise of his academic staff available to industry on a contract research basis, and already the turnover from this venture has exceeded the £1 million mark. These successes, together with the establishment of a compulsory industrial year for undergraduates, M.Sc. projects sponsored by many firms, several collaborative Ph.D. programmes and industrially supported research fellowships, are a continuing demonstration of his concept of what makes a good academic engineering department in present day conditions—that is, one where its staff are not only scholars but also professional engineers aware of the needs of the industrial world and

of where real wealth is created.

Professor Chick will be sadly missed by his colleagues and friends for his considerable intellectual qualities, his dry humour and his loyalty. Only days before his death, he visited a sick colleague in hospital carrying ice and a radio. The radio was returned as not working, and when told that the reason was that the batteries were in the wrong way round, he beamed and laughingly said, 'Failed the first term's examination!'

K. G. Stephens
T. E. Allibone

Moses Kunitz

THE death of Moses Kunitz on 24 April 1978 at the age of 90 brought to a close the story-book career of a young émigré from the Russian revolution who was to become a leading scientist in the field of protein research.

Kunitz arrived in New York early in the second decade of this century and found employment in the laboratory of Jaques Loeb, the famous experimental cell physiologist at the then Rockefeller Institute. Dr Loeb recognized the value of Kunitz's quick but careful work and encouraged him to pursue his education in the evenings, first at Cooper Union and then at Columbia University where he received his Ph.D. degree in 1923.

When John H. Northrop was appointed to the place left vacant by Dr Loeb's death, he invited Kunitz to remain and continue his study of proteins. This happy, fruitful union lasted until Dr Northrop left for California in 1951.

Kunitz was an acute observer and experimentalist. He learned to know the properties of proteins so well that he could estimate with some accuracy the likelihood of a protein crystallizing just by watching a flocculation during salt or acid precipitation. One example of this ability stands out in my mind. After attempts by other workers to crystallize the toxic protein ricin during World War II had failed, Kunitz was asked to have a try at it. I brought the package of partially purified ricin to him late one afternoon and remained to watch him weigh a quantity of it, dissolve it in dilute buffer, and distribute aliquots into a number of tubes. He then added increasing numbers of tiny drops of acid to the various tubes. As a cloudiness developed

in a few tubes prior to flocculation, he examined them carefully against indirect light, murmuring to himself 'it isn't so bad', and put the tubes in the refrigerator. The next morning crystals of the ricin protein were clearly visible in two of the tubes.

Kunitz's isolation of some 18 enzymes and precursors in crystalline form from animal and plant tissues and yeast, each with evidence of its purity went far to confirm Northrop's thesis that enzymes are proteins.

Although his name is associated primarily with protein crystallization, Kunitz's contributions and capacity were much broader. In one of his least known and yet more important papers, 'The Kinetics and Thermodynamics of Reversible Denaturation of Crystalline Soybean Trypsin Inhibitor' (*J. gen. Physiol.* 1948, 32, 241), Kunitz demonstrated by enzymatic and physico-chemical methods that renaturation of the inhibitor yielded a product indistinguishable from the original protein. Using a kinetic approach to the equilibrium reaction from both sides at different temperatures, he obtained clean thermodynamic values for each process. This study laid to rest the prevailing notion that so complicated a process as protein denaturation could not be completely reversible.

On the occasion of Kunitz's being awarded the Carl Neuberg Medal in 1957 by the American Society of European Chemists and Pharmacists, Dr Northrop wrote the following: 'Dr Kunitz possesses to a rare degree the abilities of a research worker of the first rank in his chosen field—imagination, ingenuity, and persistence, great technical skill, mathematical facility and thorough theoretical knowledge. It is not surprising, therefore, that he has been able to solve almost every problem he has attempted. Some of them are of great importance. The isolation and crystallization of ribonuclease, hexokinase, and desoxyribonuclease placed the protein nature of enzymes in general on firm experimental foundation. In addition, the nucleases have been invaluable tools in the elucidation of the chemistry of the nucleic acids, those remarkable substances that appear to be the very "stuff of life".'

Kunitz was the first to demonstrate that certain enzymes, especially the proteases of mammals, existed in glandular tissues in an inactive form. This formation explained why the tissue proteins were not digested. He went on to study the autocatalytic or catalytic conversion of these precursors to active enzymes following secretion into the digestive fluids.

To present-day biomedical investigators Kunitz may be remembered for having purified the pancreatic nucleases, ribonuclease and deoxyribonuclease which, as Dr Northrop has noted, have become and will continue to be very important in identifying the functions of the various nucleic acids.

During his lifetime, Dr Kunitz received a number of honours including the Carl Neuberg Medal and membership in the National Academy of Sciences. He was made a full member of the Rockefeller Institute in 1950 and became a Professor when the Institute became a University.

Moses Kunitz is survived by a son, Jaques, and a daughter, Rosaline Albert.

Roger M. Herriott

R. K. Murton

RON MURTON was one of the few really professional scientific ornithologists, who combined careful and patient field observation of birds with rigorous scientific investigation on their anatomy and physiology. His death from a heart attack on 12 June 1978, at the early age of 46, came as a great shock to his colleagues and friends. Although he had a previous attack some weeks earlier, and had been seriously ill, he had returned to part-time work with his enthusiasm undiminished, and he appeared to be making a good recovery. Up till this spring, he had always had such energy and drive, and the ability to work at high pressure sparing neither himself nor his colleagues, that we had never imagined that he would ever suffer from ill health.

Ronald Keir Murton was born in 1932. The first sixteen years of his working life, after graduating in Zoology at University College London, were with the Infestation Control Division of the Ministry of Agriculture, Fisheries and Food. He became immediately involved in the study of birds which are agricultural pests, and with the wood pigeon in particular. The damage done by wood pigeons to brussels sprouts and other brassicae, amounting to many millions of pounds every year, was well known, and the main attempt at control was by supplying cheap cartridges to enable farmers and others to shoot the birds. This gave much pleasure to these sportsmen, but, as Murton soon showed, it had little significant effect on pigeon numbers and it did not prevent the birds from doing economic damage. As a result of his findings the cheap cartridge scheme was stopped, saving the taxpayer a considerable sum of money, farmers whose sport became more expensive were less enthusiastic.

Murton's work on pigeons had a very practical aim, and it was successful in identifying the problems even if it was not possible to find any easy solution. Although the study was what would today be called 'customer orientated', it was also (as such studies may be) work of real scientific quality. His observations on pigeon behaviour

and reproductive physiology have seldom been equalled. The results are recorded in some 30 scientific papers, in a 'New Naturalist' monograph volume, and they formed the subject of the Barnes Memorial Lecture, which he was invited to give to the Association of Applied Biologists at the unusually early age of 32.

Although Dr Murton's career was almost entirely in research, he was closely associated with the academic world. In 1968–69 he enjoyed a year's sabbatical leave as a visiting lecturer at the University of Hong Kong. In 1974 he was appointed an Honorary Reader in the department of zoology at Hull University, and recently he was promoted to Honorary Professor. He was in demand to lecture to university students and others.

After leaving Hong Kong he returned briefly to the Ministry of Agriculture in 1969, and then transferred to Monks Wood Experimental Station, at that time under the Nature Conservancy. When the research branch of the Conservancy was reorganised as the Institute of Terrestrial Ecology he remained at Monks Wood, and was soon appointed as Head of the Sub-Division of Animal Function.

During the years when he was working on pigeons, which included important observations on feral pigeons in cities, Murton realised the importance of studies of the physiological changes in these birds as a basis for understanding their behaviour and their whole ecology. He became much involved in such topics as the photo-periodic control of the breeding cycle, and other, mainly laboratory, studies. He considered that these were the logical development of his earlier work. Many colleagues regretted his virtual abandonment of those field studies at which he had been so adept, and thought him ill advised to attach so much importance to his newer investigations. Not all his recent work has had the general approval given to his outstanding pigeon studies. Only time would have shown whether his switch in interest was, as he insisted, wise.

As well as observing birds in the field, Murton was one of the most outstanding bird photographers. As long ago as 1963 he was elected to Association of the Royal Photographic Society. During his comparatively short period, just twelve months, in Hong Kong, he made a surprisingly large collection of slides of the nesting habits of many of the rarer and more interesting species, several of which had not been previously photographed. He used his own photographs to illustrate his books and his lectures.

Kenneth Mellanby

announcements

Awards

The Fields Medal has been awarded to **Dr Daniel G. Quillen**, (Massachusetts Institute of Technology) for his work on algebraic K-theory.

The first recipient of the C D Nelson Award of the Canadian Society of Plant Physiologists, is **Dr J. Derek Bewley** (University of Calgary) for his research contributions to plant physiology.

The winners of the Filtration Society's 1978 Travel and Study awards are **G. H. Read** (Imperial College, London) and **Maureen Farrell** (Georgia). **Professor Frank Tiller** (University of Texas) has won the Society's gold medal for his work on delayed cake filtration.

Henry F. Schaeffer III (University of California, Berkeley) is the 1979 recipient of the ACS Award in Pure Chemistry.

Professor G. Vassart (University of Brussels) has been awarded the bi-annual 'Sir Charles Harrington Prize' by the European Thyroid Association for his work on the thyroglobulin messenger RNA.

The RIC have awarded the 1977 Meldola Medal and Prize to **Professor Eric Oldfield** (University of Illinois) for his work on the NMR spectroscopy of biochemical systems.

The Marlow Medal is restricted to Fellows of the Faraday Division of the Chemical Society of not less than 3yr standing on the closing date for application (1 January 1979). Applicants should be under 33 at this date. The award is based on publications on any subject normally published in JCS Faraday Transactions I and II. Applications to: Mrs Y. A. Fish, Faraday Division, The Chemical Society, Burlington House, London W1, UK.

Appointments

Professor Barbara E. Clayton to the Chair of Chemical Pathology and Human Metabolism at the University of Southampton.

Professor James R. Gray to Dean of the Faculty of Science at Heriot-Watt University.

Dr Asa G. H. Blakeley to the Chair of applied physiology at the University of Leicester.

Professor Peter G. Sammes to the Chair of Organic Chemistry at Leeds University.

Charles E. M. Yates to be Professor of Mathematical Logic at The University of Manchester.

Geoffrey Edwards to be chairman of the Thames Water Authority.

Dr Michael I. Gurr to head of the NIRD's Nutrition Department.

Meetings

25-30 November, **4th World Ozone Congress**, Houston (Dr A. Netzer, Environmental Sciences, University of Texas at Dallas, Richardson Texas 75080).

27 November-1 December, **Radiation Protection in Nuclear Power Plants and the Fuel Cycle**, Bristol (BNES, 1-7 Great George St, Westminster, London SW1, UK).

27-30 November, **Forage Conservation in the '80's**, Brighton (The Secretary, The British Grassland Society, Grassland Research Institute, Hurley, Maidenhead, Berks, UK).

28-30 November, **International Conference Antennas and Propagation**, London (The Institution of Electrical Engineers, Savoy Place, London WC2, UK).

30 November-1 December, **Cell Surfaces and Neoplasia**, London (Dr M. Moore, Paterson Laboratories, Christie Hospital & Holt Radium Institute, Manchester, UK).

30 November-1 December, **Symposium on Nutrition and Gastroenterology**, New York (Institute of Human Nutrition, Columbia University, College of Physicians and Surgeons, 701 West 168th St, New York, New York 10032).

1-2 December, **Current Concepts of Pain and its Treatment**, New York (Dr R. Duvoisin, ARNMD, Annesberg Building, Suite 14-02, 100th St at 5th Ave, New York, New York 10029).

2-3 December, **Science and Art in the 14th Century**, New York (Ann Collins,

The New York Academy of Sciences, 2 East 63rd St, New York, New York 10021).

4-6 December, **3rd Annual Toxic Substances Control Conference**, Washington (Government Institutes Inc., 4733 Bethesda Ave, Washington, D.C. 20014).

6-7 December, **Engineering and the Environment: Harmony or Conflict?**, London (The Secretary, The Institution of Water Engineers and Scientists, 6-8 Sackville St, Piccadilly, London W1, UK).

7 December, **Structure Analysis for Surfaces**, York (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

11-14 December, **Design of Hydrologic Data Networks**, Tuscon (Meetings AGU, 1909 K St, Washington, D.C. 20006).

13 December, **Microstructural Instability: its Influence on long service life**, Birmingham (The Meetings Secretary, The Institution of Metallurgists, Northway House, Whetstone, London N20, UK).

14-19 December, **9th Texas Symposium on Relativistic Astrophysics**, Munich (Prof. J. Ehlers, MPI, Fohringer Ring 6, D 8000 Munchen 40, FRG).

18-19 December, **Pulsed NMR in Solids**, London (Prof J A S Smith, Chemistry Dept, Queen Elizabeth College, Campden Hill Rd, London W8, UK).

19-21 December, **Enzyme Active Sites**, London (Dr H. C. Watson, Dept of Biochemistry, University of Bristol Medical School, Bristol, UK).

3-5 January 1979, **16th Annual Solid State Physics Conference**, Warwick (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

4-5 January, **Laser Plasma Interactions**, Bangor (Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

8-12 January, **11th Miami Winter Symposium**, Miami (UMED-PO Box 520875, Miami, Florida 33152).

14-16 January, **5th Annual Meeting of the International Embryo Transfer Society**, Denver (Mrs S Seidel, IETS, 3101 Arrowhead Road, LaPorte, Colorado 80535).

15-18 January, **2nd International Colloquium on Mars**, Pasadena (Dr C. W. Snyder, Jet Propulsion Laboratory, California Institute of Technology, 4800 Oak Grove Drive, Pasadena, California 91103).

22-23 January, **Medical and Industrial Applications of DNA**, Chicago (Robert S First Inc., 405 Lexington Ave, New York, New York 10017).

5-9 February, **International Conference on Neurotoxins**, South Australia (Dr I. W. Chubb, Dept of Human Physiology, Flinders Medical Centre, Bedford Park, South Australia 5042, Australia).

12-13 February, **Medical and Industrial Application of DNA**, London (Robert S. First Inc., 405 Lexington Ave, New York, New York 10017).

12-24 February, **World Climate Conference**, Geneva (WMO, Ave Giuseppe Motta 41, Geneva, Switzerland).

13-15 February, **Use of the Microprocessor as a Component in Measurement and Control Equipment**, Stevenage (Carole Meads, Sira Institute, South Hill, Chislehurst, Kent, UK).

21-24 February, **Hypoxia**, Banff (Arctic Institute of North America, University Library Tower, 2920-24th Ave N.W., Calgary, Alberta, Canada T2N1N4).

5-9 March, **30th Annual Conference on Analytical Chemistry and Applied Spectroscopy**, Cleveland (Mrs L. Briggs, 437 Donald Rd, Pittsburgh, Pasadena 15235).

12-14 March, **Controlled Environments Working Conference**, Madison (T. W. Tibbitts, Dept of Agriculture, University of Wisconsin-Madison, 1575 Linden Drive, Madison, Wisconsin 53706).

12-15 March, **International Cashew Symposium**, Cochin (Central Plantation Crops Research Institute, Kasaragod 670 124, Cannanore District, Kerala, India).

12-16 March, **Tunnelling '79**, London (The Secretary, Institution of Mining and Metallurgy, 44 Portland Place, London W1, UK).

14-16 March, **Thermal Characteristics of Tumors: Applications in Detection and Treatment**, New York (Conference Dept, The New York Academy of Sciences, 2 East 63rd St, New York, New York 10021).

14-23 March, **Einstein Centenary in Jerusalem** (Israel Academy of Sciences and Humanities, PO Box 4040, Jerusalem, Israel).

26-29 March, **2nd European Conference on Surface Science**, Cambridge (Dr P. M. Williams, c/o VG Scientific Ltd, The Birches Industrial Estate, East Grinstead, Sussex, UK).

27 March-6 April, **7th Edmond de Rothschild School in Molecular Biophysics on the Structure of Chromatin**, Rehovot (Dr H. Eisenberg, Weizmann Institute of Science, Rehovot, Israel).

29-30 March, **International Conferences on Spectroscopy**, Miami (Vijay Mohan Bhatnagar, Alena Enterprises of Canada, PO Box 1779, Cornwall, Ontario, Canada K6H 5V7).

1-8 April, **International Ophiolite Symposium**, Nicosia (Chairman, Geological Survey Department, Nicosia, Cyprus).

2-4 April, **Vacuum Processes in the Electronics Industry**, Brighton (Carole Meads, Sira Institute, South Hill, Chislehurst, Kent, UK).

4-6 April, **International Conferences on the History of Museums and Collections in Natural History**, London (Mrs J. A. Diment, Palaeontology Library, British Museum (Natural History), Cromwell Road, London SW7, UK).

3-5 April, **Molecular Dynamics of Polymeric Systems**, Cambridge (Dr C. W. Smith, Secretary Dielectrics Society, Dept of Electrical Engineering, University of Salford, Salford, UK).

9-11 April, **Kinetics of State, Selected Species**, Birmingham (Mrs Y. A. Fish, Faraday Division, The Chemical Society, Burlington House, London W1, UK).

9-12 April, **11th National Atomic and Molecular Physics Conference**, Liverpool (The Institute of Physics, 47 Belgrave Square, London SW1, UK).

17-20 April, **Electrostatics 1979**, Oxford (Dr J. F. Hughes, Dept of Electrical Engineering, University of Southampton, Southampton, UK).

24-25 April, **Advances in Optical Production Technology**, Teddington (Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

29 April-2 May, **Protein-nucleic acid Interactions**, Sitges (Prof. J. A. Subirana, Departamento de Quimica Macromolecular del C.S.I.C. Escuela T.S. de Ingenieros Industriales, Barcelona (28), Spain).

30 April-2 May, **Learning to use our Environment**, Seattle (Dr A. Roffman, Energy Impact Associates Inc, PO Box 1899, Pittsburgh, Pasadena 15230).

30 April-3 May, **International Conference on Malaria and Babesiosis**, Mexico (Dr J. P. Kreier, College of Biological Science, The Ohio State University, Columbus, Ohio 43210).

2-3 May, **Monitoring Toxic and Flammable Cases and Vapours**, London (Mrs R. Keiler, Sira Institute, South Hill, Chislehurst, Kent, UK).

19-20 April, **2nd Canadian Chromatography Conference**, Toronto (Alena Enterprises of Canada, PO Box 1779, Cornwall, Ontario, Canada K6H 5V7).

3-4 May, **6th International Symposium on Flammability and Fire Retardants**, Nashville (Alena Enterprises of Canada, PO Box 1779, Cornwall, Ontario, Canada K6H 5V7).

7-11 May, **1st International Colloquium on Receptors, Neurotransmitters and Peptide Hormones**, Capri (F. Fraiolo, Patologia Medica II, Policlinico Umberto I, Università, 00161 Rome, Italy).

15-18 May, **The Agricultural Industry and its effects on Water Quality**, Hamilton (Dr C. J. Schouten, Water and Soil Division, Ministry of Works and Development, Private Bag, Hamilton, New Zealand).

21-23 May, **Television Measurement**, London (Conference Secretariat, IERE, 99 Gower St, London WC1, UK).

21-23 May, **10th International Symposium on Chromatography and Electrophoresis**, Rimini (Dr Alberto Frigerio, Istituto di Ricerche Farmacologiche, Via Eritrea, 62-20157 Milan, Italy).

21-25 May, **Colloids and Surfaces**, Pittsburgh (Mrs G. Cohen, Carnegie Institute of Technology, Carnegie-Mellon University, Schenley Park, Pittsburgh, Pennsylvania 15213).

27 May-1 June, **14th World Gas Conference**, Toronto (Public Affairs Committee, 111 St Clair Ave West, Toronto, Ontario, Canada).

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7280(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF BOTANY SENIOR RESEARCH ASSISTANT

Applications are invited for a DOE financed post of Senior Research Assistant to study the behaviour of metallic pollutants in reclaimed derelict land within urban areas. Applicants should have research experience, and be interested in soil/plant relationships in difficult environments and the chemistry and biology of heavy metal pollution of terrestrial ecosystems.

The project is financed for three years from October 1, 1978. The initial salary will be £3,883 per annum.

Applications, together with the names of two referees, should be received not later than October 6 1978, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref. RV/933/N. 7219(A)

UNIVERSITY OF NOTTINGHAM DEPARTMENT OF PHYSIOLOGY AND ENVIRONMENTAL STUDIES Applications are invited for a LECTURESHIP IN PLANT PHYSIOLOGY

in which preference will be given to candidates with an interest in cellular or molecular aspects of the regulation of plant growth and development. The appointment will be effective from January 1, 1979, or as soon as possible thereafter.

Further details and forms of application, returnable not later than October 9, 1978, from the Staff Appointments Office, University of Nottingham, University Park, Nottingham NG7 2RD. Ref. No. 634. 7229(A)

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A tenure-track position is available in experimental or theoretical radio astronomy or in radar astronomy with the Department of Astronomy at Cornell University. The appointee is expected to teach one course per semester and may be associated with the National Astronomy and Ionosphere Center (N.A.I.C.). Salary will be commensurate with experience and qualifications. The position may begin in July 1979 or with the fall semester of that year (starting August 24). Applicants should send resume and have three letters of recommendation sent to Professor K. I. Grisen, Chairman Department of Astronomy, Cornell University, Ithaca, N.Y. 14853.

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Applications in U.S.A. and Canada should be sent to: The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C., 20036, U.S.A.

7279(A)



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TECHNICIAN/RESEARCH OFFICER

required at our Tumour Immunology Unit at University College to assist Dr M. Feldmann with immunological experiments involving tissue culture and biochemical techniques and some animal work. H.N.C. or Degree plus relevant laboratory experience. Salary range £3,615 to £5,256 including London Weighting. For application form and further information write or telephone Miss S. M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields WC2 on 242 0200 ext. 305, quoting reference no: 288/78. 7260(A)

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The influence of hormonal status, species and sex differences on the metabolism of organochlorine compounds.

The support is for three years with the possibility of an extension and the duties include up to ten hour demonstrating per week during term time.

Enquiries and/or applications including the names of two academic referees, should be addressed to Professor G. M. H. Waites, Department of Physiology and Biochemistry, University of Reading, Whiteknights, Reading RG6 2AJ. Telephone Reading 85123 extension 7675 (Ref. T.N. 63A). 7257(A)

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TECHNICIAN/RESEARCH OFFICER

To assist with biochemical research at our Mill Hill Laboratories NW. Experience in nucleic acid research or a closely related field would be an advantage. H.N.C. or degree plus years laboratory experience. Salary range £3,615 to £5,257 including London Weighting. For application form and further information write or telephone, quoting reference no: 286/77 to Miss S. M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields WC2 on 242 0200 ext. 305. 7261(A)

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Applications stating research experience and the names of 2 referees should be sent to Dr L. N. Johnson, Laboratory of Molecular Biophysics, South Parks Road, Oxford OX1 3P, from whom further details may be obtained. 7239(A)

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Application forms from the Staff Officer, Portsmouth Polytechnic, Alexandra House, Museum Road, Portsmouth PO1 2QQ, to whom completed applications should be returned by October 13, 1978.

Please quote Reference B36.
7276(A)

UNIVERSITY OF
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DEPARTMENT OF PHYSICS
POSTDOCTORAL
RESEARCH FELLOW

A Postdoctoral Research Fellow is required to work with a group studying nuclear disintegration using real and virtual photons in the range 10–160 MeV at the Kelvin Laboratory 160 MeV electron linear accelerator. Applicants should have several years experience of working in the field of nuclear physics or a related field. An appointment will be made for 18 months in the first instance.

Salary will be on the range 1A £3,660 to £4,365 per annum, according to age and experience.

Applications, together with a curriculum vitae and the names and addresses of two referees should be sent to Dr D. Branford, Physics Department, James Clerk Maxwell Building, The King's Buildings, Mayfield Road, Edinburgh EH9 3JZ, before November 1, 1978. Please quote Reference 5055.
7274(A)

UNIVERSITY COLLEGE
LONDON
DEPARTMENT OF
PHYSICS AND ASTRONOMY
POSTDOCTORAL
RESEARCH ASSISTANT IN
HIGH ENERGY PHYSICS

Required as soon as possible after October 1, 1978 to work on an experimental programme using the S.P.S. Accelerator at CERN, Geneva. The successful applicant will probably be based in Geneva and will help in the running of a large experiment designed to study 2-Body exclusive hadronic processes at very high energies, using large double-arm spectrometer. Salary will be in range £4,858 to £5,342 p.a. inc London Allowance plus U.S.S. If based in Geneva, fares, housing and additional allowances are provided.

Applications, giving details of qualifications and curriculum vitae and names and addresses of 2 referees, should be sent to Dr B. G. Duff, Department of Physics and Astronomy N), University College London WC1E 6BT as soon as possible.
7248(A)

CSIRO AUSTRALIA Chief of Division Division of Chemical Physics Clayton, Victoria

Applications are invited from scientists who have an established record of personal research achievement and leadership, for the position of Chief, Division of Chemical Physics. The position has become vacant on the retirement of the foundation Chief, Dr A. L. G. Rees.

CSIRO is Australia's largest and most comprehensive research organisation, having approximately 7000 employees of whom 2400 are research and professional scientists. Its broad charter covers research into problems of primary and secondary industries and also such fields of community interest as human nutrition, the environment and the development and use of natural resources. The Organization has 42 Divisions or independent Units dispersed throughout Australia.

The Organization is at present implementing the Government's decisions relating to the recommendations of the Independent Inquiry into CSIRO. Applicants may therefore wish to read the Inquiry Report; in addition they may find it helpful to read the statements made in Parliament on 11 May 1978 by the Prime Minister and the Minister for Science relating to the Government's decisions.

The Division of Chemical Physics, located in Clayton, a suburb of Melbourne, Victoria, adjacent to Monash University, has a staff of 103, which includes 56 scientists and engineers. Research in Chemical Physics was established in 1944 in the Division of Industrial Chemistry to meet the urgent demand for the introduction to Australia of major chemico-physical methods which were being rapidly developed overseas. The Division of Chemical Physics was formed in 1958. Throughout its history the Division has made contributions of a basic character to scientific knowledge, combined with development programs related to scientific instruments.

The current research of the Division is centred on a number of specialised fields, including atomic absorption and resonance spectroscopy, molecular spectroscopy, specialised optics (including optical diffraction gratings), mass spectroscopy, magnetic resonance spectroscopy, electron diffraction, electron microscopy, X-ray diffraction, solid state chemistry, crystal defect physics, theoretical chemistry, and development of scientific instruments and techniques.

A Chief of a Division is responsible to the CSIRO Executive for the development, scientific direction and integration of the research programs of the Division, and is encouraged to promote active collaboration with other Divisions, industry and various research bodies working in associated areas.

The Executive has recently reviewed the objectives, work and management of the Division of Chemical Physics, and the new Chief would be expected to take cognizance of the recommendations arising from the review.

The salary for the position is negotiable but will not be less than \$A33,052 p.a.

The terms of the appointment will be discussed with the short-listed applicants at interview. The appointee would be eligible to contribute to the Commonwealth Superannuation Scheme.

Further information on the Division is in a booklet available on request from the Secretary, CSIRO, at the address below.

Dr Hill W. Worner, a member of the CSIRO Executive, is Chairman of the appointment committee for this position and would be pleased to discuss the position with potential applicants. He would also be pleased to receive advice concerning this appointment from people with a particular interest in it but who do not wish to be considered for it. Dr Worner can be contacted at the address below.

Applications stating full personal and professional details and names of at least two professional referees and quoting reference number 582/155 should reach:

The Secretary, CSIRO, P.O. Box 225, Dickson ACT 2602, AUSTRALIA, by 31 October 1978.
7278(A)

Nature Conservancy Council Higher/Senior Scientific Officers

Salary Scales:

Higher Scientific Officer £4,101-£5,448

Senior Scientific Officer £5,154-£6,898

An allowance of £465 p.a. is also payable for the London based posts

Applications are invited for the following posts with the Nature Conservancy Council

1. Wildlife Advisory Branch—HSO/SSO based in London

The person appointed will provide the Branch with a scientific information resource on the status of individual species, their habitats and research related to them both nationally and in connection with the Branch's responsibility for the Convention on International Trade in Endangered Species (CITES) and other international matters concerning species. He/she will also provide scientific support within the Branch in the preparation and drafting of papers, replies to correspondence, initial examination of proposals on species conservation, briefing for and preparation and servicing of meetings.

2. Wildlife Advisory Branch—HSO based in London

The person appointed will provide general scientific support to the Branch and be the focus within the Branch for specialist advice on one or more species or groups, habitats and other specific areas of the work of the Branch, be responsible for the general co-ordination and oversight of the work funded by the Branch by contract and casual labour and will interpret new scientific knowledge into an action strategy where it is appropriate to the work of the Branch.

3. Scientific Development and Advisory Unit, Scotland Advisory Officer: Rural Land Use and Planning— HSO/SSO based in Edinburgh

The person appointed should preferably have experience in the field of rural land use. The main emphasis of the work will be on broad issues related to the impact of major land uses such as agriculture, forestry, fisheries and game management, recreation and tourism, and water resource development on the natural environment, with specific reference to nature conservation interests. The Advisory Officer will be required to represent the Nature Conservancy Council on inter-departmental committees dealing with issues of rural land use. A knowledge of technical practice in agriculture or forestry would be an advantage.

4. Scientific Development and Advisory Unit, Scotland Advisory Officer: Impact of Industrial and Urban Development—HSO/SSO based in Edinburgh

The person appointed should preferably have experience in the field of environmental impact studies. The main emphasis of the work will entail the development of expertise and the giving of advice to NCC Regional and Scottish Headquarters staff on the impact on the natural environment of major industrial and urban developments in Scotland with special emphasis on the energy industry, notably North Sea Oil and Gas, and allied petrochemical developments, but also including major mineral extraction and quarrying developments. This will involve close study of the technical requirements of the industries concerned and of developments in environmental protection in relation to them. Close liaison with industry and government regulating agencies will be essential.

A current driving licence is essential for posts (3) and (4)

Age and Qualifications

All candidates should have a first or second class honours degree, or equivalent, in biology, botany, zoology, geography or geology.

Higher Scientific Officers: Candidates should be under 30 years of age and have at least two years relevant postgraduate experience.

Senior Scientific Officers: Candidates should be between 25 and 32 years of age and have at least 4 years relevant postgraduate experience.

Application forms and further details are available from Recruitment Section, Nature Conservancy Council, Godwin House, George Street, Huntingdon, Cambs PE18 6BU (Tel: 0480 56191)

Closing date for receipt of completed application forms 27 October 1978.

7224(A)

THE MEDICAL RESEARCH COUNCIL OF NEW ZEALAND DIRECTOR

Applications are sought for appointment to the position of Director of the Medical Research Council of New Zealand, to replace Dr J. V. Hodge, M.D., F.R.A.C.P., who has indicated his wish to withdraw from the Directorship in mid-1979.

The Council aims to support the balanced development of medical and related biological research in hospitals, universities, or Council's own units, from funds largely provided from a Government grant. Standing committees assist Council in evaluating applications for research grants and fellowships, and provide leadership and expertise in fields of special need or concern. Administration of Council's activities is carried out at present from its office in Dunedin.

Council is seeking a person with proven experience in medical or related biological research and administration. The position is equivalent to an appointment at university professorial level.

Intending applicants are invited to write to the Secretary of the Council or to the Secretary-General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, for further information and the details required in an application. Applications should reach the Secretary of the Council at P.O. Box 6063, Dunedin, New Zealand, not later than January 31, 1979. Council reserves the right to make no appointment or to appoint by invitation.

7266(A)

CORNELL UNIVERSITY

Applications are invited for a post-doctoral research associate position in our common project, procaryotic gene expression in yeast. This position requires Ph.D. in molecular biology or in molecular genetics and research experiences with plasmids, restriction enzymes and DNA sequencing. The position will be available November 1, 1978.

Send curriculum vitae and three references to:

Dr G. R. Fink, Department of Genetics, 216 Bradford, Cornell University, Ithaca, N.Y. 14853.

Dr A. A. Szalay, Department of Biochemistry, Molecular and Cell Biology, Wing Hall, Cornell University, Ithaca, N.Y. 14853.

Affirmative action/equal opportunity employer. 7263(A)

UNIVERSITY OF BRISTOL POSTDOCTORAL RESEARCH ASSISTANTSHIP in Theoretical Chemistry/ Theoretical Physics

Applications are invited for a SRC funded postdoctoral research assistantship, available for two years from October 1978, to study the theory of photodissociation processes. The project will be aimed at developing theoretical methods for describing and predicting the outcome of chemical photodissociation processes. The work will involve the use of molecular scattering theory techniques. No prior knowledge of scattering theory is necessary, but experience computing would be desirable. Initial salary in the range £3,883 to £4,311 per annum (scale under review).

Applications, with curriculum vitae and the names of two referees, to: Dr G. G. Balint-Kurti, School of Chemistry, Bristol University, Bristol BS8 1TS, U.K. 7240(A)

THE UNIVERSITY OF MANCHESTER TECHNICIAN

required with a minimum of O.N.C. or equivalent qualification and laboratory experience to work in the Department of Obstetrics and Gynaecology, University Hospital of South Manchester. The work will involve organ culture of female reproductive tissue, histology and autoradiography. Assistance will also be required in the establishment of new research methods into human fertility. Suitable candidates may be considered for registration for higher qualifications.

Salary scale £2,688 to £3,060 p.a. Applications, including a curriculum vitae and the names and addresses of two academic referees should reach the Secretary, Department of Obstetrics and Gynaecology, Research and Teaching Block, University Hospital of South Manchester, West Didsbury, Manchester M20 8LR by no later than October 6, 1978. 7262(A)

LUDWIG INSTITUTE FOR CANCER RESEARCH (LONDON BRANCH)

(In association with the Royal Marsden Hospital and the Institute of Cancer Research, Sutton, Surrey)

RESEARCH ASSISTANT

required to undertake laboratory duties with a clinical and fundamental research programme on hormone receptors in breast cancer. Familiarity with work in this field is not essential but candidates will be preferred with two or three years proven experience in laboratory techniques and with higher qualification in Chemistry/Biochemistry. Starting salary probable within current range of £3,580-£4,680, according to age and qualifications. Further particulars may be obtained from The Secretary, Ludwig Institute for Cancer Research (London Branch), The Hadow Laboratory, Clifton Avenue, Sutton, Surrey SA5 8PX. 7236(A)

UNIVERSITY OF
MINNESOTA
COLLEGE OF VETERINARY
MEDICINE
St Paul, Minnesota, U.S.A.
DEAN

Applications and nominations are invited for the position of Dean of the College of Veterinary Medicine. Candidates must be veterinarians and should have demonstrated ability in scientific research and teaching and an understanding of the service aspects of veterinary medicine. Previous experience in practice, academic, industrial, or governmental veterinary medicine is required.

The Dean provides leadership for all aspects of the educational, research and service functions of the faculty and has budgetary responsibility for the college, which has 82 faculty members, 125 civil service personnel and approximately 320 students enrolled in the D.V.M. program and 100 students in graduate and residency programs.

Applications should include vitae and names and addresses of references and should be sent to Dr A. J. Linck, Chair, Advisory Selection Committee, 213 Morrill Hall, University of Minnesota, Minneapolis, MN 55455. They must be postmarked no later than November 27, 1978.

The University of Minnesota is an equal opportunity educator and employer. 7228(A)

ROTHAMSTED
EXPERIMENTAL STATION
Harpenden, Herts AL5 2JQ
PLANT PHYSIOLOGIST

to work in the Soils and Plant Nutrition Department on investigations into the distribution and properties of crop roots in the field, in relation to their absorption of nutrients and water.

Qualifications: First or upper second class honours degree in botany or biology, with good background in chemistry. Preference will be given to candidates with a doctorate or other postgraduate experience in the field of plant physiology or ecology.

Appointment in grade of Scientific Officer (£3,294 to £4,415) or Higher Scientific Officer (£4,101 to £5,448) starting salary depending on experience. At least two years' relevant post qualifying experience is required for appointment at H.S.O. Non-contributory superannuation.

Apply in writing to the Secretary giving names and addresses of two referees, and quoting Ref. 351 by October 27, 1978. Further particulars on request. 7233(A)

UNIVERSITY OF
STRATHCLYDE

Applications are invited from experimental or theoretical physicists with postdoctoral research experience for a LECTURESHIP in the DEPARTMENT OF NATURAL PHILOSOPHY. Applicants should have research interests related to the work of the Department in laser/plasma physics. The successful candidate will be required to participate in general undergraduate and postgraduate teaching in physics as well as in the research interests of the Department.

Salary scale £3,883 to £7,754 with placing according to qualifications and experience. Superannuation benefit.

Application forms and further particulars (quoting 49/78) and enclosing a self-addressed envelope (9"x4") may be obtained from the Academic Appointments Officer, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, with whom applications should be lodged by October 9, 1978. 7227(A)

Western Australia

MYCOLOGIST/PLANT PATHOLOGIST TO WORK ON PHYTOPHTHORA CINNAMOMI RANDS

CONTRACT APPOINTMENTS

Department of Conservation and Environment

SALARY:

\$A15,286

QUALIFICATIONS AND EXPERIENCE:

Tertiary qualifications with postgraduate research experience in soil mycology, plant root diseases or plant pathology essential. Postdoctoral experience may be an advantage.

DUTIES:

Phytophthora cinnamomi Rands is currently causing extensive damage to the jarrah forest in Western Australia. To date over 200,000 hectares of forest have been affected. The successful candidate will be expected to conduct research on the biology, epidemiology, detection and control of *Phytophthora cinnamomi* Rands. The appointee will be required to liaise and work closely with research personnel in the Commonwealth Scientific and Industrial Research Organization, the Forests Department and the Tertiary Institutions in the State under the general direction of a Supervisory Panel.

LOCATION:

Initially with Dr Andrew Glenn, School of Environmental and Life Sciences, Murdoch University, Perth, Western Australia.

CONDITIONS OF SERVICE:

The successful applicant will be required to enter into a contract with the Minister for Conservation and the Environment. The term of the contract will be for five years. Conditions applying under the contract will be the same as for temporary employees employed under the Public Service Act and include four weeks annual leave, cumulative sick leave and other general conditions applicable to temporary employees.

TRANSPORT AND REMOVAL ASSISTANCE:

The cost of first class air fares incurred by the appointee, wife and dependent children under the age of sixteen years, plus a reasonable amount for removal of essential personal and household effects, subject to the appointee entering into an agreement to remain in the employ of the State for one to three years, according to assistance given. A single appointee will receive the cost of first class air fares. Some assistance towards the cost of removal of personal effects will be granted.

APPLICATIONS:

To state age, citizenship, qualifications and experience with the names of two referees to the Chairman, Public Service Board, 111 St. George's Terrace, PERTH, WESTERN AUSTRALIA 8000.

Closing: October 27, 1978

7273(A)

Technicians

Production and Research

There are currently several vacancies at our new production and research laboratories at Milton Keynes

Senior Technician - Drug Metabolism

To carry out Metabolism Studies in animals and man. Applicants must have a good general knowledge of most laboratory techniques. Previous experience could be advantageous. Qualifications required are BSc in Bio Chemistry, Biology or related subjects; MI Biol. or equivalent.

Technician - Drug Metabolism

To assist in Drug Metabolism Studies. Experience in this area would be desirable but not essential as on the job training would be given. Applicants must have 'A' levels, HNC, HND or equivalent.

Technician - Production

To assist with processes associated with the production of veterinary bacterial vaccines. Applicants should have HNC/HND or equivalent. Experience of microbiology or of production processing would be an added advantage.

These posts carry excellent salaries, our conditions of employment are first class and our benefits package is of the standard expected from a major international company.

Please write or telephone for an application form to:

Mrs. Sheila Matthews, Hoechst UK Limited, Walton Manor, Walton, Milton Keynes. Tel: Plneham 5068.

Hoechst



7225(A)

MEDICAL RESEARCH COUNCIL ELECTRON MICROSCOPIST

Applications are invited from graduates with a degree in Medicine, or Biological Sciences to develop a research programme into the mechanism of action of toxic substances. The successful candidate will be responsible for leading a group consisting of one other scientist (to be appointed) and two technicians together with research students and visiting scientists. He will also be responsible for promoting collaborative research with other members of the unit working Toxicological problems, either in the whole animal or in isolated cells cultured *in vitro*.

Salary on MRC scientific staff scales (non-clinical) depending on age and experience.

Apply in writing enclosing a full curriculum vitae and giving the name of two referees to the Director, Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF.

MRC
Medical Research Council

7272(A)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF ATOMIC PHYSICS

Applications are invited from persons with postgraduate research experience for the post of DEMONSTRATOR in Atomic Physics. The Demonstrator would contribute to undergraduate teaching and conduct research in laser spectroscopy, high resolution spectroscopy or in experimental or theoretical aspects of atomic collisions. Well-equipped research programmes are active in each of these topics. The appointment will be for three years.

Salary will be at an appropriate point on the Grade 1B (Bar) scale £3,384 to £4,882 per annum, according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Two copies of applications together with the names and addresses of three referees, should be lodged with the Registrar, The University, Newcastle upon Tyne NE1 7RU, not later than November 6, 1978. Please quote Reference N

7277(A)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following:

FACULTY OF SCIENCE DEPARTMENT OF BIOCHEMISTRY SENIOR TUTOR IN MICROBIOLOGY

Applicants should have a higher degree and teaching interests in Microbiology. The appointee will be expected to contribute to the teaching programme and to assist in preparation, supervision and assessment of practical classes in second-year general microbiology and third-year classes in microbiology, immunology and molecular genetics. The appointee will be required to participate in training PhD and Honours students in the research of the Department (primarily in collaborative ventures with the other staff).

Research in the Department (Head: Professor J. F. Williams) includes studies of the molecular basis of motility in slime moulds, protein and nucleic acid synthesis in arbovirus-infected cells, hydrogen generation by blue-green algae, R-plasmids in enteric bacteria, *Drosophila* genetics, intermediary metabolism, membrane structure and function, lymphocyte transformation.

Appointment as Senior Tutor is normally for one year in the first instance with the possibility of yearly renewal up to five years.

CLOSING DATE NOVEMBER 17, 1978

SALARIES Salary on appointment will be in accordance with qualifications and experience within the range Senior Tutor \$A13,083 to \$A14,987 per annum. Current exchange rates \$A1=£SUS1.15; 59p.

OTHER CONDITIONS Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with housing is provided for an appointee from outside Canberra.

The University reserves the right not to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain the further particulars from the Association of Commonwealth Universities (Apsu), 36 Gordon Square, London WC1H 0PF 7267(A).

WELSH NATIONAL SCHOOL OF MEDICINE (University of Wales) DEPARTMENT OF HAEMATOLOGY

Applications are invited for a RESEARCH OFFICER

to work on ferritin variants in leukaemia. The project involves the tissue culture of lymphocytes and the purification and characterisation of white cell ferritins. Experience of preparative protein techniques is essential and a working knowledge of immunological and cell culture methods is desirable.

Candidates should hold a good degree or equivalent in a biological science and have particular interest in biochemistry. The project is funded by the M.R.C. for a period of 3 years. Salary will be on the scale for University Research and Analogous Staff Grade 1B (£3,384 to £4,882 per annum under review).

Applications, which should take the form of a brief curriculum vitae with the names and addresses of two referees, should be sent to the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN (quoting ref No H24/2/28).

The Department may be visited by appointment with Professor Jacobs (Tel No. Cardiff 755944 ext 2375), 7237(A).

**UNIVERSITY OF OXFORD
NUFFIELD INSTITUTE
FOR MEDICAL RESEARCH
POSTGRADUATE
RESEARCH ASSISTANT**

The post involves work on fetal growth and development, particularly in sheep and monkeys. Experience in immunoassay procedures preferred.

Applicants should possess a good honours degree in Physiology or related subject. The post is available immediately and is funded to December 1983. Salary on Oxford Research Staff 1B scale, £3,189 to £5,282 p.a. (subject to review).

Letters of application, together with curriculum vitae and the names of two referees, should be sent to:

The Administrator
Nuffield Institute
for Medical Research
Headley Way
Headington
OXFORD OX3 9DS

7275(A)

**COMMONWEALTH
AGRICULTURAL BUREAUX**

Vacancy for
DIRECTOR

**COMMONWEALTH
BUREAU OF
NUTRITION**

Buckburn, Aberdeen

The vacancy occurs because of the retirement of Miss D. L. Duncan, B.Sc., Ph.D.

The main work of the Bureau is to collect and disseminate to research workers information on the nutrition of man and animals and its implications for agriculture and human welfare. This is done largely through the medium of two journals (Nutrition Abstracts and Reviews Series A—Human and Experimental, Series B—Livestock Feeds and Feeding), which cover in the form of abstracts, relevant scientific papers published world-wide. The abstracts are regarded by scientists as an indispensable source of reference. Like all others in the CAB series, the two journals in machine-readable form are accessible online through the DIALOG system.

The Director is responsible for maintaining the high standards of the journals and for the day-to-day management of the Bureau.

Applicants with appropriate scientific qualifications should have had experience in agriculture, veterinary or human medicine, or in one of the biological sciences basic to nutrition. Familiarity with administration is required. Experience in editorial work and the production of scientific publications would be an advantage. A knowledge of European languages other than English is desirable.

The scale for the post is £6,609 to £9,450 per annum. The starting salary will relate to qualifications and experience. The post is pensionable.

The normal retiring age is 60, but this may be extended to 65.

Application forms and full particulars can be obtained from the Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN. Closing date: October 31, 1978.

7234(A)

For those who are seeking a change in their careers we now offer this new service

APPOINTMENTS WANTED

will appear weekly; the advertising charges are £1.50, or £3.00 per line (approximately seven words). Minimum 3 lines. Box numbers are automatically given without further charge, all correspondence to: NATURE CLASSIFIED

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**UNIVERSITY OF ASTON
IN BIRMINGHAM**

**DEPARTMENT OF
BIOLOGICAL SCIENCES
DIABETES RESEARCH**

Applications are invited for a

**POSTDOCTORAL
RESEARCH FELLOW**

to join the Experimental Diabetes Research Group.

The project concerns the preparation and efficiency of microencapsulated insulin. Chemically, biochemically or pharmaceutically trained candidates with experience of and interest in the biological applications of polymers would be preferred.

The appointment is for a period of three years and the salary will be within the range £4,382 to £4,882 per annum.

Preliminary enquiries may be made to Dr T. Atkinson (Tel. 021 359 3611, Ext. 423).

Application forms and further details can be obtained from the Staff Officer (quoting Ref. 303/6), University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET to whom applications should be forwarded not later than October 27, 1978.

7221(A)

**UNIVERSITY OF GLASGOW
RESEARCH ASSISTANT**

DEPARTMENTS OF

**SURGERY AND BIOCHEMISTRY,
GLASGOW ROYAL INFIRMARY**

Applications are invited for the post of RESEARCH ASSISTANT in an interdisciplinary group investigating the production of foetal antigens by human tumours. The post is suitable for a graduate in biochemistry or related subject. Experience in radio-immunoassay techniques would be an advantage. The successful candidate will work in the new Department of Biochemistry, Glasgow Royal Infirmary. The appointment is funded by the Cancer Research Campaign and is tenable for one year. The salary will be within the University Research Assistant scale (£3,660 to £4,130 per annum presently under revision). The appropriate superannuation scheme applies. Starting date by agreement.

Further information can be obtained from Dr J. G. Ratcliffe, Department of Biochemistry, Royal Infirmary, Glasgow G4 0SP (Tel. 041-552 3535, Ext. 3275) to whom applications including curriculum vitae and the names of two referees should be sent within two weeks of the appearance of this advertisement.

In reply, please quote Ref. No. 4241(M). 7242(A)

**UNIVERSITY OF DURHAM
DEPARTMENT OF CHEMISTRY**

Applications are invited from Chemists or Chemical Physicists for an S.R.C. Postdoctoral Senior Research Assistantship for seventeen months from as soon as possible to work in collaboration with Dr J. Yarwood on the interaction and dynamics of hydrogen bonded systems in the liquid phase. These studies which include infra-red, far-infra-red, Raman and neutron scattering studies are also supported by a N.Y.A.T.O. research grant and would be carried out at Durham, the Technical University, Braunachweig and at A.E.R.E., Harwell. Some experience in one branch of molecular spectroscopy is essential and an interest in the chemical physics of molecular motions and interaction in the liquid phase would be an advantage.

Initial salary in the range £3,660 to £4,130 p.a. (under review) on National Research Range 1A plus superannuation.

Applications (3 copies) naming three academic referees should be sent by October 6, 1978 to The Registrar and Secretary, Science Laboratories, South Road, Durham DH1 1LE. 7255(A)

MRC

Medical Research Council

NATIONAL INSTITUTE FOR MEDICAL RESEARCH

Division of Biochemistry

Applications are invited for the post of

HEAD OF THE DIVISION OF BIOCHEMISTRY

The Division at present has a staff of 31 and its main areas of activity are in cell membrane structure and function, regulation of protein biosynthesis and in ribosome and nuclear biochemistry. The future form of the Division has yet to be decided and can be discussed with potential candidates.

The post is a permanent one carrying a salary within the professorial range.

Enquiries should be addressed to the Director, National Institute for Medical Research, The Ridgeway, Mill Hill, London NW7 1AA to arrive not later than 31st October 1978.

7235(A)

UMIST

Research and Teaching Posts in
Building Science and Technology

UMIST has an active, developing programme of research into environment, services, energy conservation and production problems of buildings. Applications are invited from candidates of either sex for up to six temporary appointments which could be of up to two or three years duration in the following fields:

1. Building Services Unit

This Joint Unit has recently been established between the Department of Building and the Control Systems Centre to promote investigation into the thermal dynamics and the control performance of buildings and building environmental services.

2. SRC-supported project: Investigations of the thermal and moisture dynamics of buildings

This is a large scale project which is concerned with a number of aspects of building performance: large-scale laboratory investigations of the thermal response of buildings, heat and moisture flow in building materials, and field studies in existing buildings.

In total five research assistantships and one temporary lectureship are available. The temporary lectureship will be specifically connected with the Joint Unit. Both recent graduates and those with research experience and higher degrees are invited to apply. The posts would be of interest to graduates in, for example, Mechanical or Electrical Engineering, Physics, Chemistry, Materials Science or Building Science.

Salary for Research Assistants on Range 1B (£3,384-£4,882) or Range 1A (£3,883-£8,555) and for Lectureship on scale—£3,883-£7,754.

Application forms and further details may be obtained from the Registrar, UMIST, PO Box 88, Manchester, M60 1QD, by quoting ref. BU1/161/AI.

7238(A)

Department of Health and
Social Security

Pharmacist/Chemist

The British Pharmacopoeia Commission is based in London and is responsible for all standards published in Britain for substances and articles used in human and veterinary medicine, and collaborates in the publication of the European Pharmacopoeia. It is aided by committees comprising leading experts in the medical, chemical, pharmaceutical and allied fields.

The scientist appointed will become familiar with all aspects of the work associated with the Commission's activities and will act as secretary to a number of Committees and Panels. The work will involve the initial collection of data for the preparation of specifications to control identity, purity and potency, as well as minute writing and preparation of copy for printing.

Candidates, normally aged under 30, should preferably have a good honours degree in pharmacy, chemistry, biology or related discipline, combined with at least 2 years' postgraduate experience, and have some knowledge of the quality control of medicines.

Appointment as Higher Scientific Officer (£4560-£5910) with starting salary according to qualifications and experience. Promotion prospects. Non-contributory pension scheme.

For further details and an application form (to be returned by 20th October 1978) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote ref: SB/11/KD. 7254(A)

**Science
Group**
CIVIL SERVICE



INVERESK RESEARCH
INTERNATIONAL

RODENT TOXICOLOGY

If you are a graduate (or equivalent) with some years' experience in toxicology and are not currently earning the salary which toxicologists should command these days, or if you are ready for a career step, get in touch. We may just make you an offer you cannot refuse and might not be able to match elsewhere.

Our laboratories are situated in rural East Lothian convenient for coast and country yet close to the city of Edinburgh. The fringe benefits which are significant will include, where appropriate, a generous rota allowance, and a very substantial relocation package.

Application forms, quoting ref. 2323, are available from Miss J. M. Dunbar, Inveresk Research International, Inveresk Gate, Musselburgh, EH21 7UB, Tel 031 665 6881. 7220(A)

GROUP MANAGER R & D HEALTH MARKET

For many decades we have numbered among the well-known and successful German pharmaceutical companies whose many ethical pharmaceuticals contribute markedly to therapy. We are now in the process of intensively engaging ourselves in the health markets with the intention of making a substantial contribution to the general increase of human health through new physiological concepts and products. For this challenging undertaking we seek the Group Manager Research and Development who will lead and motivate our R & D team and provide the impetus and guidance we need to realise our plans.

The position requires a NUTRITION PHYSIOLOGIST with a scientific or medical training who possesses good personal contacts within the circles of his colleagues at universities and research institutes, is theoretically familiar with current problems in nutrition physiology, has experience in the planning and realisation of practical scientific work, and is capable of presenting new developments in the area of dietary products in a scientifically correct and pedagogically attractive form. In addition, if he has some industrial experience in the area of specialty diets and essential nutrition components, knows the relevant legal regulations and understands enough of the market situation in order to be able to speak with authority to his partners in marketing, he will be able to successfully achieve our goals.

To find the right man for this position is of crucial importance to us, we have therefore put together an appropriately attractive package. This as well as the other important details we would like to discuss with you in person. Please contact us through our consultant who will treat your inquiry in utmost confidence. Mrs R. Nicolai will be at your disposal for a first telephone contact during normal office hours under the following number:

0 1049 211-57 90 97

A letter addressed to her at the address given below will also reach us without delay. INTERELECT Unternehmensberatung, Kaiser-Wilhelm-Ring 12, D-4000 Düsseldorf 11 7256(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the temporary post of postdoctoral RESEARCH FELLOW for a fixed period of 3 years. The post is for research into the organisation and structure of the major protein species of myelin. Experience in the fields of protein chemistry or membranes would be an advantage but is not essential.

Salary in the range £3,883 to £4,882 (under review) on the 1A Scale for Research and Analogous Staff according to age and experience.

Applications, enclosing a curriculum vitae and the names of two referees, should be sent to Dr J. Findlay, Department of Biochemistry, The University of Leeds, Leeds LS2 9JT. Closing date for applications October 19, 1978. 7218(A)

UNIVERSITY OF SHEFFIELD

TECHNICIAN (Grade 5)

required for the Department of Biochemistry. The person appointed will be required to operate the University Amino Acid Analyser service. Applications are invited from graduates or non-graduates with H.N.C. Salary on scale £3,186 to £3,720 per annum.

Please write to the Administrative Officer (Personnel), (Ref. S1058/N), The University, Sheffield S10 2TN 7230(A)

THE UNIVERSITY OF MANCHESTER CHAIR OF BIOCHEMISTRY

Applications are invited for a new established additional Chair of Chemistry in the Faculty of Science. The existing Chair in the Department is held by Professor G. R. Bar. Particulars of the appointment to be obtained from the Registrar. Applications (two copies, one suit for photocopying) giving full details of qualifications, experience, research etc., and the names and addresses of three persons to whom reference may be made should be sent to Registrar, The University, Manchester M13 9PL by November 14, 1978. Quote ref 178/78/N 7216(A)

THE UNIVERSITY OF MANCHESTER DEPARTMENT OF PSYCHIATRY

RESEARCH FELLOW IN NEUROPHYSIOLOGY

Applications are invited for this post (tenable until December 1980) to study the electrophysiological consequences of adrenoceptor activation in mammalian nerve cells using intracellular recording techniques. The appointee will collaborate with a small research team working within the field of cellular neuropharmacology. Applicants should possess a Ph.D., and have research experience with neurophysiological techniques. Prefer starting date: January 1.

Salary range p.a. £3,883 to £6,500. Applications with curriculum vitae and the names of two referees should be sent before October 31, to Dr Szabadi, Department of Psychiatry, University of Manchester, Stopford Building, Oxford Road, Manchester M13 9PT. 7166(A)

VIROLOGIST

The National Institute for Biological Standards and Control is a multidisciplinary Institute concerned with the standardisation and control of biological substances used in medicine and associated research work. The Institute is a WHO International Laboratory for biological standards.

We are seeking to appoint a scientist in the Institute's Division of Viral Products to carry out research work on viral vaccines aimed at the development of improved methods for their standardisation and control.

The position provides an excellent opportunity to contribute to the active virological research programme of the Division which is well equipped for modern techniques.

Applicants should hold a PhD or equivalent qualification in medicine or veterinary science and should have recent research experience in biochemical or immunological aspects of animal virology.

Salary will be on the universities non-clinical lecturing scales.

Application forms and further details of the post are available from Peter Collingridge, National Institute for Biological Standards and Control, Holly Hill, Hampstead, NW3 6RB. Telephone: 01-435 2232, ext 211. Please quote Reference No: 0148. 7231(A)

NIBSC

National Institute for Biological Standards and Control

UNIVERSITY OF GLASGOW POSTDOCTORAL POST

Applications are invited for a post-doctoral position in an MRC-supported programme studying **Molecular and Genetic Control of Antibody Formation**. The emphasis of the group is on application of recombinant DNA techniques to molecular genetics of antibodies. Experience in any aspect of recombinant DNA or DNA sequencing methodologies would be an advantage. Position available immediately and is enable for up to five years from date of appointment.

Salary scale: £3,384 to £7,754. Applications with curriculum vitae and names of two referees as soon as possible to: Professor A. R. Williamson, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ. In reply please quote Ref. No. 233(M). 7243(A)

QUEEN MARY COLLEGE University of London CHEMISTRY DEPARTMENT Applications are invited for a GRADUATE RESEARCH ASSISTANTSHIP

or work with Professor R. Bonnett and Dr M. B. Hursthouse on the isolation and structure of bile pigments. The post, which is for 2 years, would suit candidates with experience of isolation techniques and an interest in medical biochemistry. Initial salary in grade (under review) £3,384 to £3,883 p.a. plus £450 London Allowance. Please apply by letter, giving age, qualifications, experience and names of 2 referees, to The Registrar, N, Queen Mary College, Mile End Road, London E1 4NS, not later than October 15. 7253(A)

UNIVERSITY OF ST ANDREWS LECTURESHIP IN MARINE BIOLOGY

Applications are invited for a lectureship in Marine Biology to teach in a newly instituted Honours course of Marine Biology in the Gatty Marine Laboratory of the University of St Andrews.

The course is due to start in October 1979 but the appointment will commence April 1, 1979 to allow the appointee to assist in planning and organisation. Preference may be shown for an experimentalist with research interests in plankton, but no candidate in any other field is excluded from applying.

Salary at appropriate point on scale £3,660 to £7,308 (under review), starting salary probably not above £5,282, plus F.S.S.U./U.S.S.

Applications (two copies preferably in typescript) with the names of three referees should be lodged by November 3, 1978 with the Establishments Officer, The University, College Gate, St Andrews, Fife, from whom further particulars may be obtained. 7249(A)

APPOINTMENTS WANTED

BIOCHEMISTRY TECHNOLOGIST, A.I.M.L.S.

25, with excellent academic record, seeks position in continental Europe, Australasia or North America. Prefer non-clinical field (e.g. research, industrial, publishing). Box No. 7265(B), c/o Macmillan Journals Ltd., 3 Dyers Buildings, London EC1N 2NR. 7265(B)

THE UNIVERSITY OF LEEDS DEPARTMENT OF PLANT SCIENCES

GRADE 5 TECHNICIAN IN ELECTRON MICROSCOPY

Applications are invited for the above post. The person appointed will be in charge of the maintenance and preparation work concerned with a research and teaching facility which includes three transmission electron microscopes (a Siemens Elmiskop 1A, an A.B.I. EM6B and a Siemens 102), a scanning electron microscope (Cambridge S6000) and ancillary equipment.

Applicants should possess an H.N.C. or equivalent qualification and have had about 10 years relevant experience.

Salary on the scale £3,186 to £3,720 a year.

Applications with the names of two referees and full details of qualifications and practical experience should be submitted by October 6, 1978 to The Secretary, Department of Plant Sciences, Baines Wing, The University, Leeds LS2 9JT. 7259(A)

FELLOWSHIPS

UNIVERSITY OF BRISTOL POSTDOCTORATE RESEARCH FELLOWSHIP

Applications are invited for a post-doctorate fellowship established to investigate the synaptic mechanisms regulating the peptidergic neurones of the magnocellular nuclei of the rat hypothalamus. Previous experience of electrophysiological recording from the CNS is required, and some expertise in the application of iontophoretic methods is desirable.

The appointment is funded by the Medical Research Council for two years from October 1, 1978. Salary will be determined by age and experience (minimum £4,382 per annum at age 26—scale under review).

Applications giving details of age, qualifications and experience, together with the names of two referees, should be sent by October 16 to Dr D. W. Lincoln, The Medical School, University Walk, Bristol BS8 1TD, from whom further particulars are available (Bristol 24161, ext. 557). 7241(E)

UNIVERSITY OF DUBLIN TRINITY COLLEGE Chemistry Department

Applications are invited for three postdoctoral fellowships in this Department, funded by the National Board of Science and Technology. The salary will commence at £3,762 p.a., and the appointments will be for one year in the first instance, with a possible extension for two further years. Appointments can be made from October 1, 1978.

The projects are as follows:

- In Organic Chemistry (in collaboration with Dr P. H. Boyle) to study the chemistry of quinonoid compounds used as food additives. This project will also receive support from industry.
- In Inorganic Chemistry (in collaboration with Drs C. J. and D. J. Cardin) for the study of catalytically active organometallic derivatives of transition metal cluster compounds. The project will involve synthesis, structure determination, and study of catalytic behaviour. Experience in organometallic or inorganic chemistry is required, and knowledge of, or an interest in learning aspects of X-ray crystallography would be an advantage.
- In Organic Chemistry (in collaboration with Professor T. B. H. Murray) to study the photochemical intramolecular addition of olefinic bonds to cyclic enones. Experience in synthetic and/or photochemistry would be an advantage.

Applications with curriculum vitae and the names and addresses of two referees should be sent to: The Establishment Officer, West Theatre, Trinity College, Dublin 2. 7217(E)

FLINDERS UNIVERSITY OF SOUTH AUSTRALIA SCHOOL OF BIOLOGICAL SCIENCES RESEARCH FELLOWSHIP IN BIOLOGY

Applications are invited from suitably qualified persons for a Research Fellowship tenable within any of the fields represented in the School of Biological Sciences of the University. The Research Fellowship, which is available from February 15, 1979, is tenable initially for one year but application may be made for an extension of up to a further twelve months. Applicants should have completed a Ph.D. degree prior to taking up the appointment, or have had equivalent research experience.

The appointment will be made according to qualifications and experience within the salary range \$A13,083 to \$A14,987. Persons with a Ph.D. will normally be appointed on the third step (\$A13,846). A contribution will be made towards the travel costs of the Fellow and his dependents in taking up the Fellowship.

The School of Biological Sciences is an integrated school, without subdivisions into separate departments. Research conducted in the school includes the following fields of study: Behavioural Biology (drug tolerance, neuro-transmitters and learning), Biochemistry (regulation of growth, carcinogenesis, cyclic nucleotides, control of DNA synthesis, chromatin substructure, cytokinins, phytochrome effects, aldosterone action, xenobiotic metabolism), Biophysics (energy conservation in photo-synthesis, fast movements in plants, solute transport by plant glands, vision physiology and anatomy), Developmental Biology (germ plasma in Anura, reproductive embryology, nuclear transplantation), Genetics (population genetics, radiation and chemical mutagenesis, genetics of Drosophila compound chromosomes, Drosophila development), Immunology (mechanisms of self-tolerance, and of autoimmune disease), Microbiology (control of viral macromolecular synthesis, viral pathogenesis, genetic control of recombination in Neurospora), Physiology (Animal) (locomotion, temperature and water homeostasis, responses of liver and abdominal nerve receptors to various stimuli, physiology and paleobiology of marsupials), Physiology (Plant) (structural and functional relationships between the plant embryo and surrounding tissues, drought hardiness), Plant cytogenetics (arid zone genetic systems, karyotype evolution, translocation heterozygosity), Population Biology (ecological and genetic studies), Vertebrate Palaeontology.

Applications, including full personal details, details of academic record and publications, a brief outline of research interests and names and addresses of at least two referees, should be lodged with The Registrar, The Flinders University of South Australia, Bedford Park, South Australia, 5042, by October 31, 1978. Each applicant should ask his or her referees to forward their reports directly to the Registrar before the closing date.

Further details concerning the staff of the School and their fields of interest are obtainable from the Registrar. 7269(E)

ULSTER: THE NEW UNIVERSITY

SCHOOL OF BIOLOGICAL AND ENVIRONMENTAL STUDIES

The Development of a Biological System for the Treatment and Utilisation of Whiskey Distillery Waste

RESEARCH FELLOWSHIPS

Applications are invited for three fellowships tenable from January 1, 1979 to work on various aspects of this project which is financed by the Wolfson Foundation. Applicants should either be at the postdoctoral level or be graduates with at least several years postgraduate experience.

Ref: 78/106 *Microbiologist*—This appointment will be concerned with basic research and development of the microbial system employed for treatment of the waste and the production of single cell protein.

Ref: 78/107 *Chemical Engineer*—The responsibilities of this member of the team will cover the design, supervision of construction, commissioning and operation of a pilot plant continuous flow fermenter system at the distillery.

Ref: 18/108 *Fish Nutritionist*—The role of this appointment will be to examine the nutritional suitability, toxicity etc. of the single cell protein product particularly in relation to its use as a feedstuff for rainbow trout.

Salary scale: (with F.S.S.U./U.S.S.) £3,883 to £4,133 to £4,382 per annum, and the appointments will be for two years in the first instance with possible renewal for a further year.

Further particulars may be obtained from The Registrar, The New University of Ulster, Coleraine, Northern Ireland BT52 1SA (quoting appropriate reference number) to whom applications together with the names and addresses of three referees, should be sent not later than October 20, 1978. 7226(E)

THE UNIVERSITY OF
BIRMINGHAM
DEPARTMENT OF PHYSIOLOGY
RESEARCH FELLOW IN
EXPERIMENTAL
NEUROLOGY

Applications are invited for a Research Fellowship (in collaboration with Clinical Departments) to work on a five year M.R.C.-funded project involving the study of stretch reflex behaviour in human subjects, normal and abnormal, by the application of engineering methods to human limbs. The project is directed by Dr P. M. H. Rack from whom further information may be obtained.

Applications from medically qualified graduates will be particularly welcomed, although science graduates are also encouraged to apply.

Salary will be on the Research Scale 1A (£3,883 to £6,555 plus superannuation) according to age and experience. The appointment will date from January 1, 1979 or as soon thereafter as may be arranged.

Applications with curriculum vitae and the names of three referees should be sent to the Assistant Registrar, The Medical School, Birmingham B15 2TJ by November 21, 1978. Please quote reference RF/Phy/2C78. 7247(E)

UNIVERSITY OF
NOTTINGHAM
POSTDOCTORAL
RESEARCH FELLOWSHIP
IN IMMUNOLOGY

A POSTDOCTORAL RESEARCH FELLOW is required for an investigation of the interaction between immune complexes and macrophages in man, leading to the development of methods for assessing immunopathological situations. This M.R.C. supported post will run for three years, with a starting salary of up to £3,895 (Range 1A) and U.S.S. membership.

The immunology department has recently moved into new accommodation in University Hospital with excellent facilities for research. It is also involved in undergraduate and postgraduate teaching as well as the provision of a comprehensive clinical immunology service.

Candidates should have a working knowledge of immunological methods and an interest in the medical application of their work. All enquiries and requests for application forms should be addressed to Dr R. G. Q. Leslie or Dr W. G. Reeves, Department of Immunology, University Hospital, Queen's Medical Centre, Nottingham NG7 2UH (telephone 0602-700111, extension 3510/3511). Closing date for application October 27, 1978. 7245(E)

UNIVERSITY OF
NOTTINGHAM
FACULTY OF
AGRICULTURAL SCIENCE
DEPARTMENT OF
PHYSIOLOGY AND
ENVIRONMENTAL STUDIES
RESEARCH FELLOWSHIPS
(PHYSICS/ENGINEERING)

Two fellowships are available for projects supported by the A.R.C. The first is concerned with the physics and economics of very low cost solar energy collectors for use in crop drying and the successful candidate will work with Dr J. A. Clark and Mr B. Wilton. The second project, supervised by Professor J. L. Monteith, will examine the feasibility of assessing the growth rate of crops from the spectral composition of reflected radiation, including measurements from the air.

Candidates should have a Ph.D. or equivalent experience in an appropriate branch of physics, engineering or environmental science. Both appointments are for 3 years on a salary scale commencing at £3,384 p.a.

Applications with the names of two referees should be submitted to and further details may be obtained from The Secretary, University of Nottingham School of Agriculture, Sutton Bonington, Nr. Loughborough. 7246(E)

UNIVERSITY OF GLASGOW
DEPARTMENT OF
BIOCHEMISTRY
POSTDOCTORAL
RESEARCH FELLOWSHIP

Applications are invited for the above post supported by a Cancer Research Campaign grant tenable for three years (maximum) from October 1, 1978, to work on cell interactions and intercellular communication in animal tissues. The project will investigate aspects of the structure and function of communicating junctions (gap junctions) formed between animal cells in culture and *in vivo*. Experience in developmental biology, electron microscopy or membrane protein chemistry is desirable. Starting salary in the range £3,883 to £4,382 (Range 1A of the Research and Analogous Staff scale). Further information may be obtained from Dr J. D. Pitts, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ. Applications, including a full curriculum vitae and the names of two referees, should be sent to Dr Pitts at the above address before October 20, 1978.

In reply please quote Ref. No. 4225(M). 7244(E)

LECTURES

NATO ADVANCED STUDY INSTITUTE
THE MECHANISM OF CONTINENTAL DRIFT
AND PLATE TECTONICS

at

Institute of Lunar and Planetary Sciences,
School of Physics, The University,
Newcastle upon Tyne, NE1 7RU, U.K.

March 27—April 10, 1979

An appraisal of the constraints placed on models for continental motions by geophysical and geological data.

Details by circular available November from:

Mr W. F. Mavor,
School of Physics,
The University,
Newcastle upon Tyne,
NE1 7RU, U.K.

7222(K)

MURDOCH UNIVERSITY
Perth, Western Australia

Murdoch University, Western Australia's second university, admitted its first students in February, 1975. The University is committed to taking a fresh approach to higher education, with the goal of responding directly to the conditions and needs of the late twentieth century. This is reflected in admission policies, organisational structures, programmes of study, philosophies of teaching and recruitment of staff.

The University is organised on a system of schools of study, with the Dean of each school having both academic and administrative responsibility.

Applications are invited from suitably qualified persons for the following position which is to be taken up on December 4, 1978 or as soon thereafter as possible.

SCHOOL OF MATHEMATICAL
AND PHYSICAL SCIENCES
(Dean: Dr L. R. Davidson)

POSTDOCTORAL
FELLOWSHIP—CHEMISTRY
(Ref. ENO353)

Applications are invited from suitably qualified chemists for a post-doctoral fellowship to investigate chemistry in non-aqueous solvents, in collaboration with Professor A. J. Parker. The research may be oriented towards applications of ionic solvation to hydrometallurgy, or solar cells, non-aqueous batteries and electrochemical science. However, chemists with a variety of backgrounds are encouraged to apply because a variety of basic techniques such as conductance, kinetics, polarography, calorimetry, potentiometry, spectroscopy and cyclic voltammetry are used in this type of work.

The appointment is for two years with the possibility of renewal for one further year.

Further details can be obtained from Professor A. J. Parker, Murdoch University, Murdoch, Western Australia 6153.

Salary Range: \$A13,083 to \$A14,987 per annum.

Conditions of Appointment include payment of a travelling allowance.

Applications. Two complete sets of detailed applications, quoting the appropriate reference number, including full personal particulars, details of tertiary qualifications, career history with description of posts held, area of special competence and interest, research completed or currently being undertaken. Membership of professional institutions or societies and positions of responsibility in these; list of relevant material published by the applicant, when available to take up appointment if offered and the names and addresses of two professional referees, should reach the Personnel Officer, Murdoch University, Murdoch, Western Australia 6153, by November 6, 1978. 7270(E)

STUDENTSHIPS

CITY OF LONDON
POLYTECHNIC
DEPARTMENT OF
BIOLOGICAL SCIENCES
S.R.C. (C.A.S.E.)
STUDENTSHIP

Applications are invited for a research studentship tenable for three years from January 1979 (or earlier, in the above department. We are seeking a physiologist/biochemist/pharmacologist who will join a small group studying the actions of Vitamin C on lipid metabolism in several vertebrates. Candidates should possess an upper second or first class honours degree.

Part of the research will be conducted at the J. A. Pyc Research Centre, Nr Stowmarket, Suffolk.

Further details of the research programme and application form may be obtained from Dr K. M. L. Morris, Department of Biological Sciences, City of London Polytechnic, Old Castle Street, London E1 7NT. 7268(F)

UNIVERSITY OF BATH
SCHOOL OF PHARMACY
AND PHARMACOLOGY
RESEARCH STUDENTSHIP

A recent graduate in Pharmacology or related discipline is needed to study arachidonic acid metabolism in human reproductive tissue. The project will be supervised by Dr K. I. Williams of the Pharmacology Group in co-operation with Mr D. Bamford, a consultant obstetrician at St Martins Hospital, Bath. The appointment, leading to a higher degree, will be for two years initially, current S.R.C. scales apply.

Applications with curriculum vitae and names and addresses of two referees to Dr K. I. Williams, Pharmacology, University of Bath, BATH BA2 7AY. 7223(F)

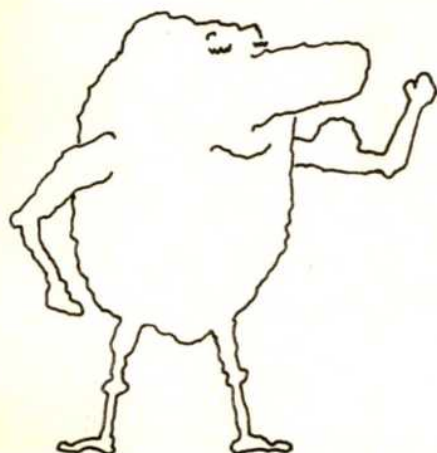
TENDERS

TENDER

East Sussex County Council
BRIGHTON POLYTECHNIC

Tenders are invited for the supply of a Rheometer of Advanced Design. Tender forms, returnable by October 6, 1978, and further details from The Chief Education Officer, P.O. Box 4 County Hall, Lewes BN7 1SG (Ref: SUPP/PGCT). 7271(T)

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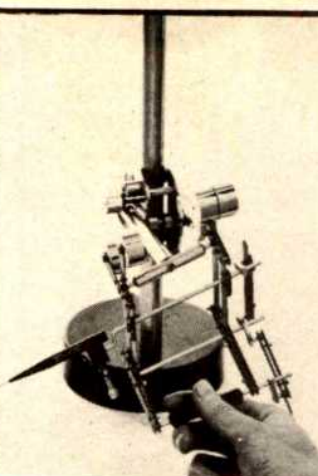


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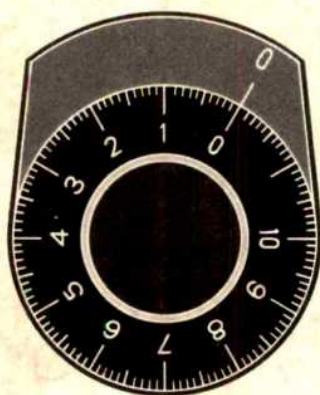
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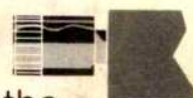
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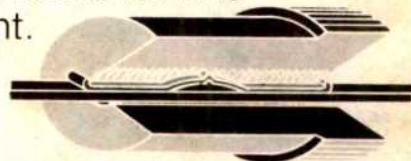
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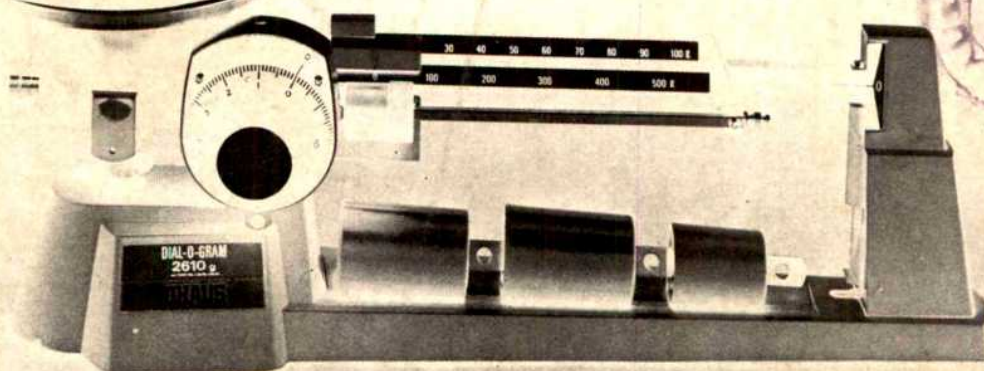
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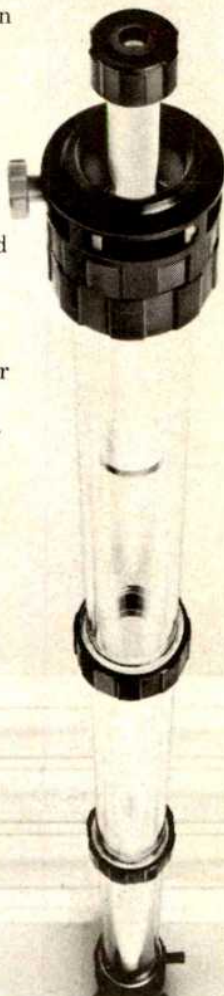
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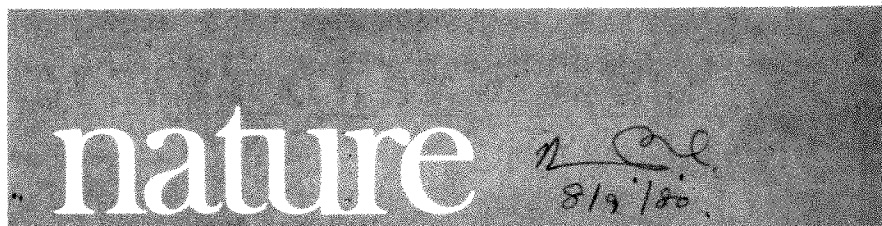
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Cover picture

Jupiter viewed in ultraviolet light.
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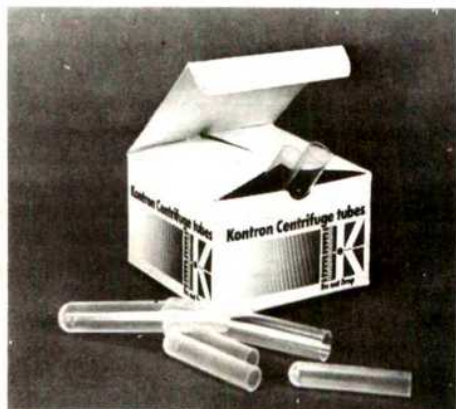
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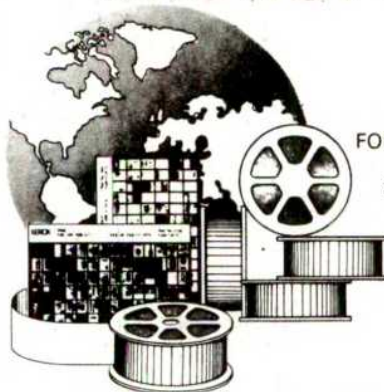
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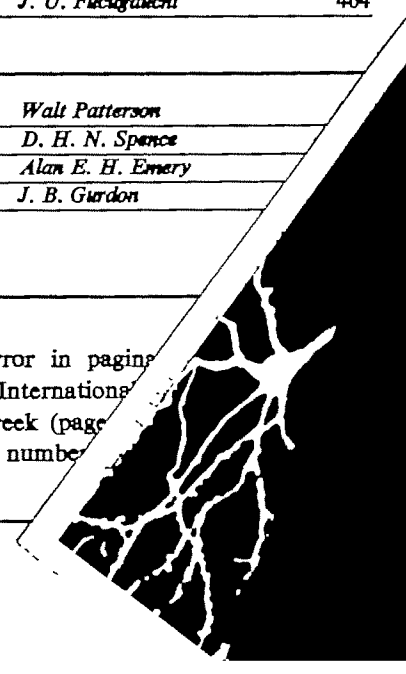
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Erratum

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Europe should invest more in space science

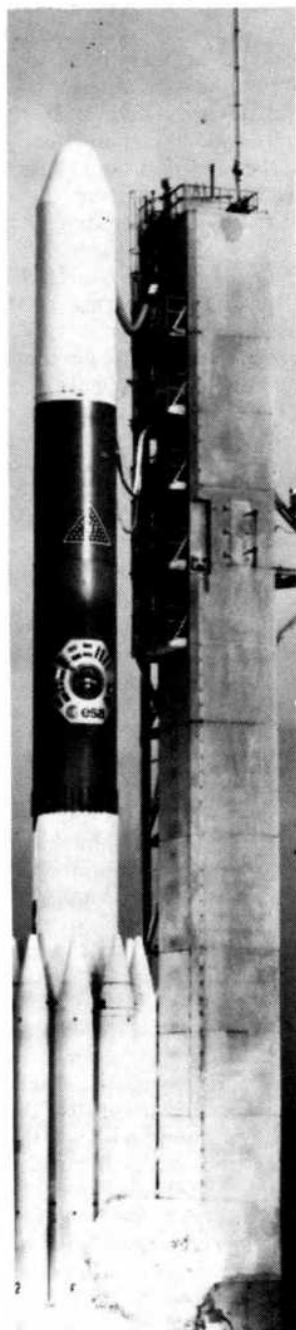
SPACE science is expensive. NASA's science budget amounts to half-a-billion dollars a year. No individual European country could afford a national programme to satisfy the needs of its astrophysicists and geophysicists to do experiments in space, let alone provide opportunities for scientists working in other fields to realise the potential space has to offer. For Europeans, therefore, international collaboration is essential. It pools funds, expertise and ideas.

The European Space Agency (ESA) undertakes this task. If ESA did not exist, space scientists in individual European countries would have to rely on *ad hoc* international collaborations. More likely than not, most of these would involve NASA, which has a world lead in space science and can build spacecraft more cheaply and efficiently than any European country. Such collaborations could undoubtedly benefit all taking part: previous ones have proved this.

But total reliance on NASA could be dangerous. For example, some European scientists might want to build a mission which is of no interest to NASA at the time it is proposed. This might not be because it is unworthy of development but simply because it is not NASA's policy to build that type of mission at that time. European scientists need some independence and ESA should be able to provide it.

This is not to say that ESA itself should shun collaboration with NASA. On the contrary, wherever such collaboration is the best way of achieving the scientific aims of a particular project, it should be done. And this applies just as much to collaboration between individual countries and NASA.

If ESA is to provide European scientists with a certain amount of independence, it must provide them with enough launches. Many European scientists feel that, because of its appallingly low budget for science—about a tenth that of NASA—and poor value for money, ESA is failing them. And matters are unlikely to improve. The few funds which are available might well be spread even more thinly in future.



ESA is now under pressure to support missions to the planets. And it will soon have to decide how to pay for materials and sources, such as climatology.

Although it cannot support some of these scientific disciplines at the expense of the others, ESA must make sure that it does not lose the support of its best scientists. There is a danger that when a group of space scientists in Europe develops a world lead in its discipline, it will want to break away from ESA because ESA cannot give it the opportunities it needs to maintain that lead.

The key to ESA's future success is more space opportunities more often. This could be achieved if member states agreed to increase the mandatory science budget. Matters could be improved even further if ESA could cut costs.

ESA's Science Advisory Committee is arguing that some of the surplus money which will be available in the early 1980s—when the applications programmes start to cost less (see page 356)—should be diverted to science. But space science is not high on the list of priorities in most member states, who want an immediate financial return for their investments.

Good science, as always, has its intrinsic merit, but there are extrinsic reasons for a substantial investment in space science. In many industries, a strong programme of scientific research stimulates the development of new technologies. This applies *a fortiori* to the space industry. Building spacecraft for scientific research often calls for greater technological advances than building applications satellites. For example, ESA's faint object camera for the Space Telescope has put Europe's industry in the forefront of advanced photon detector technology.

Further, if the development and running of space applications is taken over from ESA by industry, as seems possible, further investment in space science will be the one in which national governments can keep space industries on their toes. Space science should not be seen as a luxury but as a necessary part of a healthy industrial development.



INSIDE: eight-page special on the European space science programme

0028-0836/78/10

Scientists press ESA to restore budget to 1971 levels

ESA's future is in the balance. The end of the decade set aside for developing space applications is in sight. At the end of this year, the ESA Council will have to make some fundamental decision on the future.

Exactly how scientists should influence that future was discussed last week at a meeting of ESA's Science Advisory Committee (SAC) in Nice. "Now is the time to say that science needs more support from the member states", is how Professor Klaus Pinkau, a member of the SAC summed up the conclusion of the meeting.

The SAC would like to see some of the funds now spent on applications diverted to science in the future. In 1971, ESA's science budget was cut to make room for the new space applications. Now that many of those applications are nearing the end of their development there is a strong case for

some of the money which will be released to be spent on science.

The other factor which distinguishes ESA's present predicament from the past is how to incorporate the 'new' fields of materials and life sciences and earth resources into the science programme. It is essential that the new fields should at least be screened for their scientific worthiness by ESA's scientific advisory bodies. If they are funded outside the mandatory science budget the situation could arise where a project in one of the 'new' fields would be funded, even though scientifically it was not as worthy as a geophysics or astrophysics experiment, simply because a separate budget had been set aside for it.

The West German government is likely to support the scientists' view. It has already said that it would like to divert funds from applications to



science. The French government, however, might need some hard persuasion from its scientists. And in the UK, diverting funds from industry to science is likely to produce many administrative difficulties because of the way it pays its ESA subscriptions.

The SAC will present its ideas to the Science Programme Committee (SPC) in November. At the end of the year, it will report to the ESA Council outlining its ideas for the future. □

What the critics say

EUROPEAN space scientists are far from content with the service they receive through the European Space Agency (ESA). According to Professor Martin Rees, who until last year was chairman of ESA's Science Advisory Committee, ESA's science budget is only about a tenth of NASA's. And many claim that ESA spends it inefficiently.

ESA was created in 1975 out of the European Space Research Organisation (ESRO) and the European Launcher Development Organisation (ELDO). The former had been solely responsible for space science: the latter for developing an independent launcher capacity for Europe. Since these two objectives have been under the umbrella of one agency, there have been fears that too much attention is being paid to the industrial programme (it takes 85% of ESA's budget) at the expense of science. According to one West German official "science developed a good reputation in ESRO, but ESA is not taking enough care of it". In 1971 ESRO defined a 'minimum viable budget' which amounted to only two-thirds of its expenditure, and ESA has kept it since.

The scientists' dissatisfaction is fuelled by the frustration of some ESA's staff who feel that ESA is responsible for many ills for which it cannot be held entirely responsible. They see the exceedingly difficult task of

trying to implement a European science programme within the constraints of a very limited budget (about £50m per year, compared to CERN's £190m for high energy physics). And in recent years the reduction in many national programmes has put an even greater burden on that budget.

So far all ESA's scientific missions have been paid for out of the mandatory science budget. Each of the 11 member states (Belgium, Denmark, France, Germany, Ireland, Italy, the Netherlands, Spain, Sweden, Switzerland, and the UK) contributes to the mandatory budget in proportion to its gross national product up to a maximum of 25%.

But as time goes on, European space scientists need larger space missions more often because they need to build on the previous research of European and national missions. On top of that, scientists in fields which have never been catered for by a European space mission, in particular planetary science and climatology, are putting pressure on ESA to provide them with opportunities. And looking further to the future materials and life scientists might well be applying similar pressure if they gain their entrée into space through the launch of a manned laboratory—Spacelab in 1983. According to one West German scientist "a multiplicity of disciplines are now knocking on the mandatory science budget's door".

The difficult position ESA is in is illustrated in the long term planning

reports produced by its astronomy and solar systems working groups in 1976. They both concluded that a basic programme in each of their respective fields over the next ten years would take up the entire mandatory budget available over that time.

It is also claimed that ESA is inefficient. In recent evidence to the Committee on Science and Technology, US House of Representatives, Dr Wolfgang Finke, speaking in his capacity as Head of Space and Transportation of the Bundesministerium für Forschung und Technologie (BMFT), said that a conservative estimate of the cost of an international project "is more or less equal to the product of the 'true' costs of the project done nationally and the square root of the number of participating countries". For ESA with eleven member states this rule implies very expensive projects indeed. However, a direct comparison of the costs of ESA's satellites and NASA's or those built in an individual country, is very difficult because of differences in accounting and charging overheads. Nevertheless ESA is in the process of making such a comparison.

A major factor contributing to high costs is ESA's industrial policy. The policy was created to help develop the European aerospace industry and states that industrial contracts have to be distributed throughout member states in proportion to the size of their contributions to ESA's subscription. Thus an ESA satellite may be built in

Report was compiled
by Redfearn

several firms in several member states. There is little or no competition for contracts and therefore little natural price control. Also because space contracts are few and far between, and are only small fry to the aerospace industry, they suffer from lack of continuity and standardisation.

Standardisation could only be introduced if ESA built a series of similar satellites. However, it is unlikely that enough satellites could be built with the available funds to make standardisation a reality. In the early days, ESRO attempted to build a standard bus, but after the first, TDI, a uv satellite launched in 1972, it had to scrap the idea. "European missions have never been suitable for a standard bus" says Mr Roy Gibson, ESA's Director-General. Nevertheless, Dr Edgar Page, Head of the Space Science Department at ESA's technology centre, (ESTEC), in Noordwijk the Netherlands, believes that ESA's future success will depend to a large extent on whether or not it can achieve some sort of standardisation. At the moment, ESTEC engineers are looking at standard subsystems rather than standard buses, but these are more likely to find uses in applications satellites rather than scientific ones.

Reusing spacecraft might be another way of cutting costs. At the moment NASA's Space Shuttle is the only vehicle capable of retrieving or refurbishing spacecraft in orbit, so the cost-effectiveness of doing this will be very dependent on its launch costs and precisely how it is used. So far ESA is participating in two future reusable missions: Spacelab and NASA's Space Telescope. Spacelab, however, will probably be very expensive to operate, one of the reasons being the high cost of using the Shuttle. NASA is quoting \$31m for a Space Shuttle launch by non-US government users.

Some of the missions ESA has already flown and many of the ones it is considering for the future are too large and expensive for any individual country to build. Mr Ernst Trendelenburg, ESA's Director of Scientific Programmes, believes that these are precisely the kind of missions ESA should be adopting. Mr Gibson, however, believes that ESA should also provide smaller facilities. These differing views are reflected in the scientific community itself. Largely, big launches mean high energy astronomy, and a rather small community of users, and small launches mean geophysics and more users, except for certain very sophisticated experiments.

Until 1972, scientists needing small payloads carrying experiments of short duration were catered for by ESA's sounding rocket programme. This was stopped, however, on the grounds that

sounding rockets are within the scope of national programmes. Now ESA only helps out in the running of ESRANGE, a rocket range in Sweden. The main hope for the future in catering for small payloads is Spacelab. But there are fears that it might be too expensive to be used to best effect. "We had hoped Spacelab would be a seven day balloon or rocket facility", commented one German scientist "but it looks like becoming a seven day satellite facility". And for atmospheric scientists, at least, it will not be as useful as sounding rockets because it pollutes its environment and its orbit is uninteresting to them scientifically.

ESA's scientific satellites have become more complex as time has passed. In astrophysics, for example, early satellites such as TDI were designed to survey the sky at various wavelengths and identify interesting objects. Follow-up satellites need to observe those objects with greater pointing accuracy and higher resolution. Large



observatories in space are needed to do this. The instruments for them often have to be built under the direction of ESTEC because they are beyond the technological capabilities of universities and research institutes. Many scientists feel that this trend is taking away their opportunities to design and build instruments which are just as important for maintaining a strong scientific community as being able to do theoretical work from satellite data. ESA is developing a will of its own.

One possibility for overcoming this problem is to build large observatories within consortia of institutes. Professor R. Lust of the Max Planck Society in Munich and the first scientific director of ESRO, believes that this is possible. "EXOSAT (ESA's X-ray satellite to be launched in 1981 on Ariane) is a large observatory satellite and it is being built in industry under the direction of ESTEC. Cos B (ESA's cosmic ray satellite launched in 1975) was just as complicated and it was

built successfully in five different institutes".

Not all scientists are so critical of ESA. In spite of its shortcomings some think it provides an essential service. Professor van de Hulst of Leiden University, the Netherlands, for example believes that "ESA suffers from the problems of a big international organisation but it must be judged on the projects it has done. These have been good." And Professor Roger Bonnet, of the Laboratoire de Physique Stellaire et Planetaire, near Paris and chairman of the SAC thinks that it offers a certain independence for Europe. "Cooperation with NASA can be difficult", he says "because NASA won't always be willing to adapt its programmes."

Inevitably, the least discontented scientists are those in countries with an active national programme. Holland, for example, has a small but world-renowned astrophysics community which has been able to take advantage of opportunities on some ESA satellites, such as Cos B, and on other satellites in collaboration with other countries such as the NASA-Dutch X-ray satellite ANS-1 and the NASA-Dutch-UK infrared satellite, IRAS due for launch in 1981. It is just beginning to consider what kind of satellite to build after IRAS. Two possibilities are an X-ray satellite and another IR satellite.

Perhaps the country where ESA's problems are posed most acutely is the UK. Unlike most other European countries, its contributions are paid by two agencies out of two separate budgets. The Science Research Council (SRC), which pays about £8m, is responsible for science and the Department of Industry, which pays about £30m, for applications. The SRC therefore is a focus for scientific opinion within the UK government. In 1975, during a period of financial stringency, the SRC adopted the policy of squeezing funds for big science. Before then the UK's national space programme exceeded its contribution to ESA. Now it is slightly less and current national programme will to an end next year after the of UK-6, an X-ray satellite, some £9m. Apart from satellite, to which the UK a small contribution, the other approved international organisation outside ESA ing rocket programme to the bare bones the SRC is contributing for money it.

It is the community which is left out. In the end, the money is spent by ESRO and

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further opportunities to help them maintain the world lead they gained after UK-5, an X-ray satellite launched in 1975. They feel that the data from EXOSAT, ESA's large X-ray observatory, will be spread on the ground too thinly to be of much use to them. "The future of British astrophysics does not lie with ESA" commented one British high energy astronomer.

Recently the SRC has been devoting some thought to ways of providing UK scientists with more space opportunities and of cutting the cost of space science. It is considering collaborating with NASA on a multi-disciplinary refurbishable satellite (MRS) to be launched and refurbished by NASA's Space Shuttle. The idea is to increase the lifetime and usefulness of a single satellite by repairing it and altering its payload either in orbit or on the ground. The SRC hopes that an MRS could be a very cheap way of doing both astrophysics and geophysics experiments. There are some fears, however, especially among geophysicists that by trying to please everyone it will please no one.

The SRC would like to involve ESA in studies on the MRS, but enthusiasm for the idea within ESA is low. There are doubts about its feasibility and worries that it would mean yet another collaboration with NASA. Nevertheless, ESA will at least be obliged to look into the idea although it could not be studied in time for ESA's next project decision at the end of 1979.

West Germany's policy is to conduct as much of its space science through ESA as possible and it would actually like to increase its contribution to ESA's mandatory science budget. However, it could only do this if all member states agreed to increase their contributions accordingly. In the long term, Germany would like to see funds diverted to science from applications. "Applications should only go on in ESA for a short time," says Dr H. Strub of the BMFT, "then they must be marketed and taken out of ESA's control. In science ESA runs and develops satellites but in applications, the users must take this over once the technology has got off the ground". In particular, Germany would like to see more spent on science when ESA's own launcher, Ariane, and Spacelab near the end of the most expensive phases of their development. By the mid-1980s, it would like ESA's entire budget to level off at 350 mau (about £230m) at today's prices (it is now about 530 mau, £345m) and most of it to be spent on science and new fields to be explored on applications and instruments such as remote sensing and geophysics.

The greatest pressure on ESA to do more space science comes from

Germany. German scientists would like to consolidate the experience they gained from the Helios probes, two solar physics missions launched in 1974 and 1976 in collaboration with NASA. The French would also like to build on their collaborations with the Soviet Union on a number of planetary missions. Germany is also considering collaborating with NASA on Robi, a cheap X-ray satellite.

Scientists within the SAC feel many of ESA's problems could be solved if member states agreed to increase the mandatory science budget and ESA could increase its cost-effectiveness. Professor Klaus Pinkau of the Max Planck Institute für Extraterrestrische Physik recently sent out a questionnaire to European space scientists canvassing opinion on ESA. "The science budget doesn't need a six-fold increase" Professor Pinkau told *Nature*. "This would be out of proportion to the funding of the rest of science". He would like to see the mandatory science budget restored to its pre-1971 level, about 100 mau (£65m) at today's prices, and would like ESA to make efforts to increase its cost-effectiveness by at least 20%. Over a ten year period, he says, ESA

should be able to fly one mission a year if it built five missions costing 50 mau each, four costing 120 mau and one very good and expensive one costing 250 mau.

To achieve this, however, ESA would have to make special efforts to keep the cost of small satellites within the 50 mau limit. Professor Pinkau suggests that facilities on the ground could be used more efficiently so that the science budget does not have to pay for facilities which are not being used for some part of the year. "Good management should show where you lose money", he says. The industrial policy might also have to be revised so that the principle of fair return would apply over say ten years.

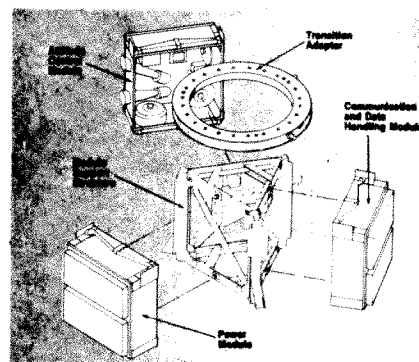
This scheme, he feels could incorporate materials and life sciences into the mandatory budget. Earth resources would have to remain outside the budget but could still be refereed within the procedure of the SAC. Such a scheme however is dependent on the member states agreeing to increase the science budget and on ESA achieving a substantial reduction in costs. The former lies within the power of national governments. The latter is up to ESA. □

The multi-discipline refurbishable satellite

THE MRS is one country's (the UK's) attempt to find more flying time outside the auspices of ESA. Under consideration by the Astronomy, Space and Radio Board of the Science Research Council, it would use The Godard Space Flight Center's kit-form Multi-mission Modular Spacecraft (MMS) as a base (see figure) and erect on it instruments to serve both astronomers and geophysicists. A study is underway (it will report in January) at British Aerospace, Stevenage, to assess the difficulties of combining the instruments on the same vehicle. "And it doesn't make life easy" said one scientist.

The MRS would be a collaboration with NASA, which is interested in flying high energy astronomy experiments. But NASA is not concerned with flying geophysics on this mission; so geophysicists in the UK are suspicious that the exercise is one designed by high energy astronomers to 'sell' the project to the Science Research Council, which is conscious of the need to serve as many scientists as possible.

Funds would be needed by about the end of 1980, for a launch from the Space Shuttle in 1985. The UK would assemble the satellite, at a cost of £15-£20m—twice the cost of UK6, for example. But the MRS could take a payload of up to 1,000 kg, and could



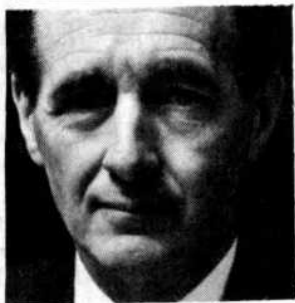
Exploded view of MMS basic modules and elements

have a continuous data rate an order of magnitude greater than UK6.

Atmospheric physics may be the casualty of the multidiscipline confrontation, as its requirements are least compatible with astronomy. But the MRS is retrievable and refurbishable and is designed to make use of the supposed reduced costs of reuse of the MMS base and some instrumentation. Geophysics could get a better look in on a subsequent launch.

But everything depends on whether an agreement can be reached with NASA on the first and subsequent flights—as always with such collaborations—and, ultimately, on the uncertain cost of operations with the shuttle. □

THE ORGANISATION



Roy
Gibson
ESA's
Director-
General

Who makes the decisions?

FINAL approval for ESA's scientific projects is granted by the Science Programme Committee (SPC), the body responsible to the ESA Council for running the science programme within the financial limits set by the Council. Each member state is represented on the SPC by its own delegates and is allowed one vote in any decision.

Projects for final selection are put to the SPC by the Director-General, Mr Roy Gibson. He is advised by the Science Advisory Committee (SAC) and four working groups: the astronomy working group (AWG), the solar system working group (SSWG), the materials sciences working group (MSWG) and the life sciences working group (LSWG). Experts appointed by the Director-General and acting in their own personal capacity, sit on each advisory body. The MSWG and LSWG were recently created. The two areas of science they serve are new to ESA. One life scientist, one meteorologist and four representatives of the astrophysical and geophysical sciences sit on

the SAC. Its meetings are attended by the chairmen of the working groups.

The executive recommends that new project proposals are screened by the working groups. The money spent on early studies is authorised by the Director-General.

If preliminary studies are successful, the Director-General may recommend that a project be considered by the SPC. The SPC will then decide whether or not to go ahead. When a project has been approved, ESA puts out a call for proposals for experiments.

Although there is no formal link between the working groups and the SAC, in practice they work together quite closely. Typically, out of 10-20 project proposals put before the working groups, 5-6 will be passed on to the SAC and three on to the SPC. On average the SPC chooses one major project for development every one and a half to two years.

So far, the SPC has only approved one experiment in the materials and life sciences, the Sled for Spacelab, to be funded out of the mandatory science budget. The rest of the experiments approved in these disciplines are for the first Spacelab payload and are being paid for in individual countries and out of the Spacelab budget.

As well as vetting individual project proposals, the working groups and the SAC discuss overall policy for the science programme. In 1976, for example, the AWG and SSWG both produced reports on the science and projects needed in their respective fields up to 1990. About once every four years, the SAC also discusses plans for the future, such as it did last week.

Responsibility for seeing that the science programme is put into practice

rests with Mr Ernst Trendelenburg, Director of Scientific Programmes. His responsibility now includes future science programmes. To speed up decision-making, he would like to see the number of early studies cut, especially those for projects which are obviously unsuitable for further development from the outset. □

Four steps to get a project off the ground

A SCIENTIFIC mission proposal which has been studied by the relevant working group and found scientifically worthy will go through four 'stages' before it is finally approved and the spacecraft is built. Each stage is independent of the others so that the mission can be scrapped at the end of any one without breaking contracts or jeopardising the money which would be spent in the subsequent stages. Most projects which go beyond phase A, however, are completed. The steps in a mission's development are as follows:

Mission definition or assessment. This is a study stage to define the mission and its payload. It is done under the direction of ESA by seven to ten scientific experts in the mission's field of study. They measure the interest shown in the mission by the scientific community in Europe, assess its scientific soundness and the ability of European universities and institutes to build instruments capable of achieving its scientific objectives. They also predict what sort of system will evolve, how

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The cost: where the money comes from, where it goes

ESA's TOTAL budget for 1978 is about 530 mau (one mau is roughly US \$1.3 million). Of this, 75.5 mau is to be spent on the science programme, about 80 mau on the general budget and the rest on Spacelab, Earthnet and applications programmes such as telecommunications satellites, Meteosat and the development of ESA's own launcher, Ariane.

The science budget and part of the general budget are mandatory. Each member state contributes to them in proportion to its gross national product. Thus, in 1976, Belgium contributed 3.48%, Denmark 2.26%, France 21.20%, Germany 25.25%, Italy 13.23%, the Netherlands 5.34%, Spain 4.44%, Sweden 4.46%, Switzerland 3.56% and the United Kingdom 16.47%. So far all ESA's scientific missions have been conducted through the mandatory programme, even

though, in principle, nothing excludes a group of interested member states from funding an optional one.

The mandatory part of the general budget funds 'basic activities' such as the technological research programme at ESTEC, the salaries of some headquarter's staff, the documentation centre at Frascati and the infrastructure. In 1978 it amounted to about 54 mau. The rest of the general budget is for facilities which cannot be charged to any particular programme such as the cost of buying the agency's headquarters in Paris and the cost of running the Ariane's launch pad at Kouru in French Guiana.

All of the science budget is spent on building spacecraft and very large instruments such as those for Spacelab or large observatory-type free-flyers, and on maintaining facilities for tracking them and receiving data. Most of

the experiments on board satellites, however, are built in universities and institutes and are funded out of national funds for scientific research.

In 1971 the mandatory science budget was fixed at 28 mau. In 1978 that level is 75.5 mau and in 1979 it is expected to be about 79 mau. As scientific satellites cost between 50-100 mau, and some of them even more, this level of funding means that ESA can build one every one and a half to two years.

The capital cost of launches is not the only expense, and already the science budget is almost fully accounted for until the end of 1980. It is proving difficult to find the 2 mau needed to keep Cos B, ESA's cosmic ray satellite launched in 1975, operating for another year. This sum has not already been included in next year's budget because Cos B has exceeded its expected lifetime. □

much it will cost and what sort of effort in terms of manpower and technological research will be needed. The study normally takes three months.

Phase A or study stage. Those proposals not thrown out after the assessment stage are subjected to a more detailed definition. This is usually done under the direction of a technical study team which may contract out to industry some parts of the study. It involves a detailed estimate of the cost and time to completion and a thorough description of the payload, spacecraft, orbit, ground support and data handling and analysis. Typically the study stage lasts nine months and costs 1% of the final project cost.

Phase B or design stage. A scientific project which enters this stage has usually been approved for development by the SPC. It is put under the management of a project team which will be responsible for it until it has been launched and is operating. The project team places contracts in industry for the design of the spacecraft. Typically, phase B takes one year and costs 10% of the final cost of the project.

Phase C/D or development and production. Typically, for a scientific satellite, this will take three years and cost 40–70 mau.

The project teams include engineers from ESTEC's department of development and technology (DDT). When they are not part of a project team they do research in one of the DDT's divisions on various aspects of advanced space technology. This research is important for keeping Europe in the forefront of space technology.

Before launch all ESA satellites are tested at ESTEC. It has the only facility in Europe—a high vacuum chamber 14 m high and 10 m in diameter—large enough to test a satellite with its booms deployed or the nose cone of Ariane. Once the satellite is in orbit, however, operational responsibility for it shifts to the European Space Operations Centre (ESOC) at Darmstadt, West Germany. As well as control of the orbit, ESOC is responsible for setting up ground stations to receive data.

One of ESA's objectives is to encourage development of European industry. This is reflected in the way it puts out tenders to industry for its scientific satellites. Its industrial policy states that each member state should receive the same percentage of the total contract put out to tender as it contributes to the mandatory science budget. This is irrespective of whether it is participating in the scientific payload. This policy is controlled by the Industrial Policy Committee (IPC) which can suggest how contracts should be placed to ensure that each country gets a fair return. □

ESA's SCIENTIFIC RECORD

The past . . .

ESA and its predecessor ESRO have launched twelve scientific satellites since the first launch in May 1968. Six of them are still operating: Cos B, a highly successful gamma ray observatory launched in April 1975; Geos 1, which failed to reach geostationary orbit in April 1977 but is producing some useful magnetospheric data; ISEE-2, which was launched in October 1977 and is observing the interaction of the solar wind and the Earth's magnetosphere in conjunction with NASA's ISEE-1; Meteosat, launched in November 1977, contributing to the Global Atmospheric Research Programme and the World Weather Watch; International Ultraviolet Explorer, the ultraviolet astronomical observatory, launched as a NASA-UK-ESA collaboration in January 1978; and Geos 2, a 'copy' of Geos 1, successfully launched into geostationary orbit in June 1978 to observe the magnetosphere.

Of the previous seven satellites, five were designed for magnetospheric and atmospheric experiments, and two for high energy astronomy: ESRO-II for cosmic rays and solar X-rays, launched May 1968) and TD-1 (a UV satellite launched March 1972 and operational for two years but troubled by malfunctioning tape recorders). Of the complete list of thirteen satellites, nine were for Earth-related observations (mostly on Sun-Earth interaction) and four for astronomy. Astronomical satellites were launched in 1968, 1972, 1975 and 1978.

The present . . .

ESA's report to the 21st COSPAR meeting in June 1978 says that while Sun-Earth relationships "continue to be an important feature of the agency's scientific programme", there is a change afoot: "an increased emphasis is being placed on astronomical studies". This is reflected to some extent in the current list of approvals (four main projects, two of which are astronomical), and to a greater extent among the competing proposals for the next project (three out of five are for astronomy). Approved are:

The first Spacelab payload. Spacelab is scheduled to fly with the tenth flight of the Space Shuttle on 3 December 1983. It will be manned, but will stay aloft for only 7–30 days. According to ESA's 1978 report to COSPAR "the composition of a typical Spacelab payload will be distinctly different from

that of a conventional research satellite". Life sciences and materials sciences will benefit from 'look-see' experiments in a new environment. But the observing time is too short for much astronomy.

EXOSAT. This 0.1 keV to 50 keV X-ray astronomy satellite is scheduled for launch on Ariane in February or March 1981 (or by Thor-Delta if Ariane is not ready). It has two operating modes: occultation, using the Earth or Moon to determine the position and structure of X-ray sources, and arbitrary pointing, which will be used to determine this spectral and temporal variation.

Out of Ecliptic/Solar Polar Mission.

This is a joint mission with NASA to launch two spacecraft in February 1983, to fly out of the plane of planetary orbits and around the North and South poles of the Sun. The gravitational field of Jupiter will be used to swing the spacecraft out of the ecliptic. The objective is to extend into three dimensions our knowledge of the solar corona, the solar wind, the solar magnetic field, the cosmic ray flux, and interplanetary/interstellar neutral gas and dust.

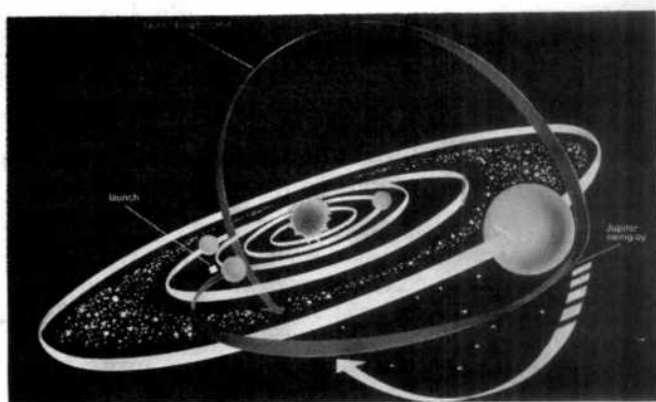
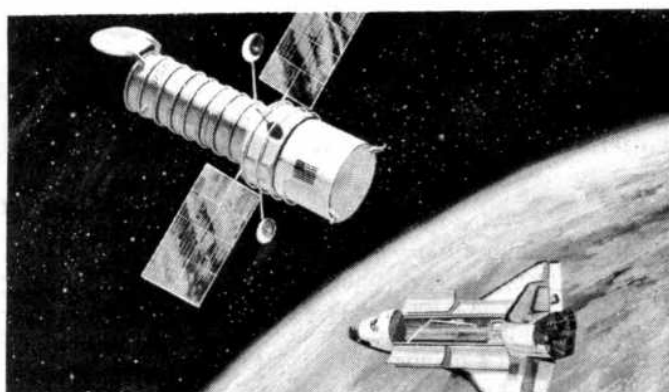
The space telescope. This, as ESA's latest report to COSPAR says, "is the most ambitious project in space astronomy currently planned and will dominate astronomical research for the rest of the century". ESA is participating at a level that will allow Europe's astronomers just 15% of the observing time on the high-resolution, 2.4 metre telescope. The telescope should be launched from the space shuttle at the end of 1983 with five separate focal plane instruments. In a 500-km high orbit, it will be retrievable and refurbishable, and should serve astronomers for two decades.

The future . . .

THE European Space Research and Technology Centre (ESTEC) in the Netherlands is investigating the scientific and technical merit of several spacecraft which will compete for selection as the scientific project to follow the ESA/NASA space telescope which is due for launch in 1983.

To effectively use the science budget ESA's Science Policy Committee (SPC) will have to decide on which experiment to launch by the beginning of 1980. The next mission should then be ready for launch in 1985.

The options are already known. According to Dr D. E. Page, head of the science department at ESTEC, any new mission proposals could not be studied in time for next year's decision



Already approved: space telescope, to be launched from the NASA shuttle, and the Out-of-Ecliptic mission, both scheduled for 1983.

because available staff at ESTEC are already committed. The selection will, therefore, be from the following:

Extreme ultraviolet and X-ray survey satellite (EXUV). The proposal for this satellite stemmed from observations made in 1975 by NASA's X-ray satellite, Copernicus. Before that it had been thought that extreme UV radiation was too heavily absorbed by the interstellar medium (ISM) to be observed. Copernicus, however, showed that the local ISM is thinner than had been expected and that in certain directions the emissions can be observed from objects up to 100 parsec away.

ESA's EXUV satellite would survey the sky in the waveband 15 Å–1,000 Å with a few arc min resolution. It would provide information on very hot stars, which emit most of their radiation in the EUV waveband (250 Å–1,000 Å), and stellar coronae. It would also help study the origins of the diffuse XUV (15 Å–250 Å) background.

The estimated cost on completion of an EXUV satellite is 75 mau. This includes half an Ariane launch.

Astrometry satellite. The position of stellar objects can be measured with far greater precision from space than from Earth. In principle, positional accuracy could be improved by a factor of 20 and the number of observable stars could rise by a factor of 10 to 100.

The idea for an astrometry satellite in ESA is about nine years old. However, it has never been one of the candidates for final selection because previous studies have been abandoned at the mission definition stage. One of the problems is that it could be technically very demanding to achieve the claimed increases in accuracy. Because of this, an astrometry satellite could be expensive, of the order of 95 mau.

Grazing-incidence solar telescope (GRIST). This would be an ESA-funded Spacelab facility to observe the Sun in the soft X-ray and UV wavebands (100 Å–1,700 Å). The advantage of using Spacelab (rather than flying GRIST separately) is that the telescope could be flown as one of several solar telescopes, making simultaneous obser-

vations in a variety of wavelengths. NASA is considering building the other telescopes.

The option of another large Spacelab facility, the large infrared telescope (LIRTS), has recently been shelved on cost grounds. Interest in focal plane instrumentation for LIRTS, however, is still being maintained at ESTEC in case the project is ever reconsidered as a free-flyer. The major disadvantage of putting a heavy instrument on Spacelab is that the launch cost for a 7–30 day flight is as much as the cost of launching it as a free-flyer with an observing time of several years.

Sun-Earth observatory and climatology satellite (SEOCs). This would measure the Earth's radiation budget more accurately than previous NASA satellites. It would help investigate the physical processes controlling the budget by monitoring spatial and spectral changes in the incoming and outgoing radiation. In particular it would monitor the incoming solar UV flux in conjunction with the composition of the stratosphere and observe interactions between different atmospheric levels and the Earth's surface.

SEOCs would be put into circular orbit at a height of 1,150 km and 57.5° inclination. It would only be able to improve on existing measurements if it were to interact with geostationary satellites, such as Meteostat, to obtain information on the diurnal characteristics of the atmosphere. It would also have to interact with polar orbiting spacecraft if it were to study the complete radiation budget. Because the usefulness of SEOCs would depend so heavily on it interacting with other spacecraft, ESA is looking at the possibility of building two.

The cost on completion of one satellite plus the cost of half an Ariane launch is estimated to be at least 85 mau. However, ESA has not yet decided whether to fund climatology out of the mandatory science budget or as an optional applications programme. So SEOCs could be removed from the list of candidates for the next scientific mission and be funded separately.

Polar orbiting lunar observatory (POLO). In spite of NASA's Apollo and Lunar missions, there are still fundamental questions about the Moon left unanswered: for example does it have a core, what is its internal temperature, what is its thermal history, and what was the origin of the magnetic field of the early Moon? Some scientists feel these questions would be better answered with the aid of an orbiting spacecraft than a lander, such as Apollo, which is necessarily very limited in the area it can observe.

A study is currently being rushed through at ESTEC to look at the possibility of building POLO very cheaply from Geos 3 hardware and launching it on the fourth Ariane test flight at the end of 1980. This would mean that POLO could be built cheaply and quickly. It might even mean that it could be funded in addition to one of the other options. The possibility of this happening, however, has grown more remote as cost estimates have risen from a few mau to well above 10 mau.

The SAC will not have to decide which project should follow the one to be approved at the end of next year until the end of 1980. ESA, however, will be sending out a call for mission proposals for that decision in the middle of next month. No one yet knows what that will bring but there is growing pressure from planetary scientists in Europe for ESA to launch spacecraft out of Earth orbit to other parts of the solar system. And POLO is not the only idea they have for the future. They are also considering a cometary mission and a mission to the asteroids. At a workshop organised by ESA earlier this year, European scientists came out in favour of a dual mission to comets Halley and Tempel 2. The proposal they agreed on was to fly-by Halley's comet during its next nearest approach to Earth in 1986 and then, following an Earth swing-by, to rendezvous with Tempel 2 in 1988. Both the proposed cometary and asteroid missions would be extremely expensive. The cost for the cometary mission might be about 500 mau. □

Astronomy in the 1980s

Astrophysics and geophysics are ESA's two oldest disciplines. Professor A. P. Willmore, of the University of Birmingham's Space Research Department presents the case for launching sophisticated equipment to do high energy astronomy . . .

EVEN more than for most sciences, the last two decades in astronomy have been a period of rapid technological development for all its branches. The substitution of high quantum-efficiency detectors for the photographic plate in optical telescopes, the application of large aperture synthesis telescopes in radio astronomy and the entire histories of infra-red, ultra-violet, X-ray and γ -ray astronomy are contained within the last 20 years. The application of space-flight to astronomy, on which the possibility of the newest branches of astronomy (only excluding work in the infra-red) has depended and which promises some important advances in the older branches too, has also occurred during this time.

The last two decades have also been a quite exceptional period of discovery whose many results now demand exploitation. It is also most unlikely that the phases of technological development and discovery are yet at an end. Thus charting a course for astronomy, especially space astronomy which is so costly, will require careful selection from a broad spectrum of projects, any of which in less exciting times might have seemed suitable to be pursued.

Space astronomy—for the obvious reason again of its expense—is highly internationalised; most experiments or facilities involving international teams with members from both sides of the Atlantic. The degree of internationalisation exceeds that even in nuclear physics so that a distinctively European programme is hard to conceive and it is useful only on a world scale to identify the promising paths to develop. But several branches of astronomy are alive and well in Europe, particularly in the UK, and there is no reason to doubt that UK astronomers will be well represented in world class astronomy, provided of course that funding at a minimum necessary level is available.

The sensitivity of large optical telescopes has been substantially enhanced in recent years by the development of photo-electric detectors whose quantum efficiency is far higher than that of photographic plates and whose performance in other respects is comparable. However, the ability of a telescope to detect and resolve objects which are very distant and therefore faint and of small angular diameter depends also on its effective image quality and on the brightness of the night sky. The angular resolution of any good, large optical telescope is determined not by diffraction or the quality of its construction but by the atmosphere, and consequently the Space Telescope, a 2.4m. orbiting telescope being built by NASA with ESA as a minor partner, will have an effective resolution 10 times and a sensitivity 100 times that of the large ground-based telescopes.

The Space Telescope can operate over a wide wavelength range, and will be an ideal instrument for the study of the most distant galaxies which are of course those which are observed in the earliest epochs in the Universe, closest to the time of the Big Bang, and around the epoch of galaxy formation. The measurements of the motions and parallaxes (the apparent motions due to the Earth's orbital motion about the Sun, on which ultimately all astronomical distance scales depend) and of astronomical objects are severely limited by atmospheric effects, and either with the Space Telescope or with satellites constructed for the purpose, improvements of about an order of magnitude should be attainable.

The short history of X-ray astronomy is especially remark-

able for the profusion of new discoveries, for example the close binary sources containing compact stars such as neutron stars and—one supposes—black holes, which allow properties—the mass, at least—of the compact star to be determined in a number of cases. It is salutary to recognise the relative crudity of the techniques which have generally been employed in making these observations, which are about equivalent to carrying out optical astronomy with something between a photographic light meter and a box camera. But much technique development has been carried out in the last 10 years, and the new techniques are now ready to be exploited. Later this year the NASA HEAO-B satellite should be launched with a medium size grazing incidence telescope. This will have an angular resolution roughly two orders higher than any previous X-ray instrument, comparable for the first time with that of an optical telescope. The sensitivity too will be far higher than that of any previous instrument—indeed it will be much better than that of any present X-ray sky survey, which will undoubtedly prove some limitation on its use. This telescope is likely to be able to detect the X-ray emission from clusters of galaxies at distances approaching those of the most distant quasars, thus providing another means of observing the early Universe. Other means of imaging at higher X-ray energies have also been developed, and this is a whole field that is sure to prove productive.

Recent observations of X-ray spectra, which like the spectra of radio sources are dominated by continuum emission, have shown the presence of measurable line emission in a wide variety of sources. This generally arises from highly ionised atoms, usually of Fe, which are present in the source, which must be at a high temperature since it is an X-ray emitter. A high energy feature, however, is believed to be cyclotron emission from around a neutron star, thus allowing for the first time a direct measurement of the large magnetic field (about 10^8 T) around these objects. The diagnostic value of such detailed spectrum observations is very high and together with much improved observations of the spatial structure of sources this is bound to prove an area of important development.

X-ray observations are generally considered to extend up to the electron-positron annihilation radiation at 512 keV. Observations of the γ -ray sky at higher energies are relatively scanty, depending on the surveys carried out with spark chambers on the SAS-2 and Cos-B satellites. Whilst X-ray and optical objects such as the Crab nebula and the Vela pulsar are detected at γ -ray energies, there is a good chance that the typical γ -ray source will prove to be qualitatively different from those observed at lower energies and promising new observational techniques such as drift chambers and double Compton scatter telescopes are now in existence.

In selecting the projects of the future, the obvious criteria to use (and one implicitly accepted in the examples already cited) will be the extent to which they are likely to contribute to fundamental topics—either cosmology, or fundamental physics as general relativity or the properties of nuclear matter. But throughout high energy astronomy, better surveys will be vitally important in the next decade, and will surely bring to light many new types of astronomical object. It will be worth remembering that even at lower energies, both pulsars and quasars were discovered within the last 20 years or so. □

Science in the solar system

... and Dr Alan Johnstone, of the Mullard Space Science Laboratories, poses some of still-unanswered questions about the solar system.

WITHIN the consultative structure of both the European Space Agency (ESA) and the UK Science Research Council, space science is divided between committees at the boundary of the solar system. Although this is a neat arrangement, solar-system science encompasses four almost completely distinct scientific fields, requiring different observational techniques and theoretical approaches. In this article it is not possible to do more than give some idea of the vigour of the research and indicate the principal directions it is taking.

Space plasma physics is concerned with the interaction between charged particles and magnetic fields. The solar wind plasma flowing outward from the Sun forms magnetospheres around the magnetised planets. The magnetospheres contain populations of high temperature plasma confined in the magnetic field.

The particle distributions and the magnetic field configuration of the Earth's magnetosphere are now well-known, but they are not enough to explain the first order operation of the magnetosphere as a plasma machine. The solar wind not only forms the magnetosphere; it is also its principal energy source. The energy is accumulated in the magnetic configuration of the magnetosphere and then released in an explosive event called the magnetospheric substorm, whose most obvious manifestation is the polar aurora. There are many unanswered questions about this process. How is the energy of the solar wind coupled into the magnetosphere and what controls the rate? How is the energy stored and what triggers its release?

The interpretation of satellite observations in such a dynamic system is hampered by the ambiguity between time variations of the whole system and spatial variations along the satellite orbit. The problem can be overcome by deploying a number of satellites in carefully coordinated orbits. This approach has been taken by the ISEE project which has one satellite stationed permanently in the solar wind and a pair of satellites moving close together through the outer magnetosphere to investigate the interaction between the solar wind and the magnetosphere. With the GEOS satellite in geostationary orbit they form a powerful combination to study magnetospheric dynamics.

Another fruitful approach is the comparison of solar system magnetospheres. Each one is dominated by a different effect: Jupiter by its rapid rotation; Mercury by the greater solar wind strength near the sun; and Mars by its atmosphere. The Earth's magnetosphere contains the elements in a dynamic balance. There will be an increase in the use of the plasma in the magnetosphere for plasma physics experiments, especially from Spacelab. The advantages include stable, fully ionised plasma, with no walls to influence the experiment.

The second field is the study of planetary atmospheres. There are now routine observations of the Earth's lower atmosphere for meteorological purposes; and there have been continuing investigations in the upper atmosphere in the regions accessible to satellites. But there have been relatively few observations of the stratosphere and mesosphere in between. A major thrust of atmospheric research is now being directed at the atmosphere between 10 km and 100 km through the international Middle Atmosphere Programme. Our ignorance of this part of the atmosphere became apparent when questions were raised about the influence of various pollutants—such as Freon from aerosol

cans and the exhaust of high-flying aircraft—on the ozone layer.

The chemical reactions which control the composition of the middle atmosphere are extremely complex. Much more information is required about the spatial distribution of various chemical species, the temperature structure, winds, waves and tidal motions before the controlling processes can be understood. All the available platforms will be used in this essentially exploratory work: aircraft, balloons and sounding rockets for in-situ measurements; and free-flying satellites and Spacelab to make global surveys by remote-sensing techniques.

As with magnetospheres, investigating the atmospheres of the other planets gives insight into the origin and evolution of the Earth's atmosphere as well. The two Pioneer Venus space probes will soon be returning detailed information about the dense and hostile Venusian atmosphere.

Solar physicists have made substantial use of space vehicles to study radiation from the Sun at wavelengths from UV to X-ray which do not penetrate through the atmosphere. Their next major project is NASA's Solar Maximum Mission, with 2 European instruments, which is designed to study solar flares during the next maximum of sunspot activity.

The scientific problems are similar to those of magnetospheric substorms. Energy is stored in the magnetic field of Sunspots and then suddenly released in the acceleration of charged particles. Similar questions need answering: how is the flare triggered, and how is the energy transferred to the particles? Since both flares and substorms seem to belong to the same class of plasma processes, any advance in one could assist the other and could also be relevant to other astrophysical and laboratory plasmas.

A second mission, the joint ESA/NASA Solar Polar Probe is going to look at the Sun from a different angle and study the three-dimensional structure of the solar wind. Two spacecraft will use the gravitational field of Jupiter to swing into heliocentric orbits normal to the ecliptic plane taking them over the north and south poles of the Sun respectively.

Finally, the field which has given some of the most spectacular results is the exploration of the planets themselves. US spacecraft are now en-route to Venus, Jupiter, Saturn and Uranus. So far the only European involvement has been by individual scientists but there are projects being discussed now which will be open for possible European participation.

I began by suggesting that the four disciplines within solar system science were grouped together just for administrative convenience. It is true that so far there has been little interaction from them. That may be because the links which must exist have not been fully recognised, but there is an extremely important unifying theme. Each field is concerned with one aspect of the physical environment which makes life possible here on Earth. Why has life developed here and apparently not on the other planets? Is there an important balance which we could unwittingly upset? The tremendous progress that has been made towards understanding all aspects of the origin and evolution of the solar system in the twenty years of research in space science, and the simultaneous realisation that human activities can have damaging effects on a global scale makes it clear that it is a field of research that we ignore at our peril. □

news and views

Insect molecular biology on Crete

from Walter J. Gehring

THIS summer biologists working on the molecular and genetic aspects of insect development converged on the island of Crete for an EMBO workshop held at Kolymbari followed by an international conference organised by the Hellenic Society for Biological Sciences in Heraklion. Crete was chosen because a new University is being established there and also to provide a stimulus to Greek biology. The meetings had a very special ambience which in part was created by the places at which the discussions were being held, which ranged from an ancient church and a Venetian fortress, to a night club and the beach. In the friendly atmosphere generated by our Greek hosts, the open exchange of information proved profitable for all the participants.

From the vast amount of new information presented in Crete only a few highlights can be mentioned.

Structural and functional organisation of the genome

The structural and functional organisation of the genome was the centre of discussion for much of the time. Among the unique genes, the silk fibroin gene has been cloned in bacterial plasmids (Y. Suzuki, Carnegie Institution, Washington) and analysed in detail, especially near the 5' end of the coding region, where one might expect the regulatory signals for transcription and processing of the fibroin messenger RNA. The 5' end of the gene was determined by hybridisation to mRNA which was specifically labelled at the 5' end, in the cap structure. The flanking sequences in front of the gene are AT-rich and contain sequences complementary to the 3' end of 18S ribosomal RNA. Like many other eukaryotic genes, the fibroin gene also contains a large intervening sequence some 1,300 nucleotides long, which is not present in the mature mRNA. Interestingly enough, the DNA sequences at the junction between the coding and intervening sequences are very similar in the mouse β -globin, mouse immunoglobulin gene and the silkworm

fibroin gene, which suggests that the enzymes involved in splicing the mRNA have conserved their specificity in the course of evolution. The notion that fibroin is coded for by a single gene is supported by recent genetic evidence (K. Sprague, Oregon). Genetic variants have been found which differ in the molecular weight of the fibroin. These variants show normal Mendelian inheritance as expected for a single gene, with codominant expression in heterozygotes. Since the protein and presumably also the gene sequence is internally repetitious, these variants in chain length are thought to be due to unequal crossing over.

Several research groups are focusing on the heat shock genes in *Drosophila*. *Drosophila* cells respond to a brief heat treatment by activating a small group of approximately nine gene loci which is reflected in the giant chromosomes by the appearance of specific heat shock puffs. These puffs are sites of intense RNA synthesis and accumulation of RNA polymerase. Under heat shock conditions transcription and translation of the remainder of the genome is reversibly turned off. Pre-existing polysomes disintegrate, a new population of polysomes carrying the newly synthesised heat shock mRNA appears in the cytoplasm, and a set of about eight heat shock polypeptides is synthesised. Obviously, regulation occurs both at the transcriptional and the translational level. This system has considerable potential for studying the coordinate control of a small group of genes. Several heat shock genes have been cloned. In particular, the genes coding for the major heat shock polypeptide of 70,000 daltons have been analysed. Both the analysis of deletions for the major heat shock puffs and evidence from *in situ* hybridisation with cloned DNA indicates that several genes coding for this polypeptide are located at two chromosomal loci, 87A and 87C, where the two major heat shock puffs are located. The restriction maps of the coding sequences at 87A and 87C are similar but not identical. The flanking sequences are very different. Heteroduplex mapping and cross hybridisation studies between the

sequences at 87A and 87C indicate homology of the coding regions and of two small regions apparently outside the coding sequences, near the 5' end of the genes. These sequences are obvious candidates for carrying regulatory signals.

The histone genes of *Drosophila* represent a tandemly repeated gene family (D. Hogness, J. Kemp, Stanford). The repeated element contains five histone genes in the order

H3 H4 H2A H2B H1,
← → ← → ←

where the underlining arrows indicate the direction of transcription of each gene. Such a cluster of five genes is separated from the next cluster by an AT-rich spacer of 1,200 base pairs, the entire element occupying five kilobase pairs. There are approximately 100 repeated elements which, however, do not form a single array, but are instead distributed among several arrays, separated by larger array spacers. Since the histone genes are present in several adjacent bands in the giant chromosomes, the subdivision into arrays may possibly reflect the band-interband organisation. The repeated element containing the five histone genes has been almost completely sequenced. Of particular interest are the short regions between the coding sequences for H2A and H2B, and between H3 and H4. The intergenic sequence between H2A and H2B spans 220 base pairs and contains a sequence of eleven base pairs which is present twice in opposite polarity. This sequence may be a promoter sequence, or part of a promoter.

Another gene family which has been studied extensively is that which codes for the chorion (eggshell) proteins of silkmoths (F. Kafatos, Harvard and Athens). In *Antheraea polyphemus* there are approximately 100 different chorion proteins synthesised and secreted by the follicle cells surrounding the oocyte in a 2-day period during egg development. Each protein is produced during a defined time interval *in vivo* and also in organ culture, indicating that the expression of the chorion structural genes is strictly regu-

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lated according to a developmental program. The proteins can be divided into a few groups A to E. The proteins within each group share homologous amino acid sequences, but differ from each other by amino acid replacements, deletions or insertions. The difficulty of purifying individual mRNAs was overcome by constructing DNA sequences complementary to the mRNA mixture and cloning a pool of hybrid plasmids containing cDNA plasmids for individual messages. Sequencing of some of the cDNA clones revealed previously unsuspected homologies between limited regions of proteins from group A and B. The sequence data suggest that blocks of DNA sequences may be rearranged between genes in the course of evolution. The arrangement of these genes in the chromosomes is not known, but genetic data in the silkworm *Bombyx mori* indicate that the genes for all four classes are located on the second chromosome and probably are clustered (M. R. Goldsmith, University of California, Irvine). The transcription of these genes is being analysed by hybridisation of *in vivo* synthesised RNA to cloned cDNAs using a newly developed spotting procedure.

Transposable genes

Among the most intriguing genes are the dispersed repetitive gene families such as *copia* and *412* in *Drosophila* (D. Hogness, M. Young, Stanford). The *412* and *copia* elements are approximately 7.2 and 5.2 kb in length, respectively, and are found at 20 to 40 different chromosomal sites. The adjacent sequences are different at the different chromosomal locations. These sequence elements are homologous to abundant poly (A)-containing cytoplasmic RNAs. A curious feature of both families is that the elements are flanked by direct repeats of 0.5 and 0.3 kb respectively. The function of these flanking repeats is not known but by analogy with bacterial systems, one can speculate that these elements are translocated to different positions in the chromosomes by reciprocal recombination events which involve the repeated termini. This hypothesis can be tested in a case in which a *copia* element occurs within one unit of a tandemly repeated sequence, by comparing the sequence of the unit with and without the *copia* element. Preliminary evidence obtained by restriction mapping is consistent with the model. Evidence for transpositions of these elements at least on an evolutionary time scale comes from the observation that two wild-type strains differ with respect to the chromosomal location of these elements.

From genetic experiments, 'jumping genes' have been known for some time

RNA processing

from Andrew Travers

THE excision of intervening nucleotide sequences during the production of eukaryotic mRNA and tRNA molecules has recently highlighted the biological importance of post-transcriptional processing. This excision together with postulated subsequent ligation often involves the removal of an RNA sequence at least several hundred nucleotides long.

How do the processing enzymes achieve the exquisite accuracy that is a prerequisite for the maintenance of the continued function of the mature RNA molecules? One possible prokaryotic solution to this problem has recently emerged from the studies of Young and Steitz on the production of the 16S rRNA (*Proc. natn. Acad. Sci. U.S.A.* **75**, 3593; 1978). This rRNA is cotranscribed with the sequences for the 23S and 5S rRNA and is generated by RNase III cleavage of the precursor followed by secondary trimming. The RNase III cleavage sites are contained in the sequences flanking the 16S rRNA gene. By determining the DNA sequences of these regions Young and Steitz have discovered that the proximal and distal sequences are potentially capable of hybridising with each other to form a stem consisting of 26 base pairs. The individual strands of this stem are of course separated by a loop of about 1,700 nucleotides containing the entire 16S rRNA gene. The biological importance of such a structure is given credence by the observation that both proximal, and distal RNase III cuts are contained within the base-paired region. Further, RNase III may require a double helical region of at least 9 base pairs both 5' and 3' to the point of scission, a structure which is lacking in the immediate neighbourhoods of the scission sites. Thus the most probable interpretation is that a site for RNA processing can be generated by the interaction of distantly spaced complementary RNA sequences. The authors suggest that an analogous process may operate during the production of

eukaryotic messenger RNAs

Cleavage by RNase III does not by itself generate the normal 16S rRNA sequence, yielding only a 17S precursor. For production of the mature 16S RNA two or more additional processing enzymes are required. One recent addition to the family is the endonuclease RNase M16 which cleaves the 16S precursor immediately proximal to the normal 5' end of the 16S RNA molecule (Dahlberg *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 3598; 1978), while yet another enzyme is apparently required for the production of the mature 3' end (Hayes & Vasseur, *Eur. J. Biochem.* **61**, 443; 1976).

A further quirk in the enzymology of RNA processing is provided by the discovery that RNase P, first detected as the activity required for the production of *su⁺* tRNA (Altman & Smith *Nature new Biol.* **233**, 35, 1971) contains a specific RNA moiety 350 nucleotides long in addition to the expected protein (Stark *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 3717; 1978). As normally purified this highly unusual enzyme contains about 80% RNA by mass. Furthermore the RNA component seems to be essential for nuclease activity. This requirement for the RNA molecule could be trivial. For example, it might be needed to stabilise an active conformation of the protein component. Alternatively the authors suggest the biologically more interesting possibility that the RNA molecule may itself participate in the recognition of the RNase P cleavage site by a mechanism involving direct RNA-RNA interaction. Implicit in such a model is of course the potential for varying the substrate specificity of the enzyme by simply changing the sequence of the RNA moiety. Again, if this enzyme is of universal occurrence such a capability might have important implications in the production of eukaryotic RNA species.

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in *Drosophila*. For example, several transposable elements carrying the *white* locus have been described. *White* was the first mutant to be found in *Drosophila* and 'traditionally' it maps on the X chromosome. However, fly stocks have been found in which the *white* locus is transposed to different chromosomal sites and transpositions are observed in these laboratory stocks. At the meetings, the isolation of a

hybrid plasmid containing complementary sequences to such a transposable element was reported (W. Gehring, Biozentrum, Basel). The element carries both the *white-apricot* allele and the neighbouring *roughest* gene. This plasmid is obviously of great interest for the study of transposable elements in *Drosophila*. It may also provide a general tool for the isolation of genes with no known biochemical function.

Since the transposable element causes mutations by inserting into genes, the flanking gene sequences can be isolated by using the hybrid plasmid complementary to the transposable element as a probe. Alternative methods for gene isolation in general were discussed, in particular the possibility of starting from a DNA sequence which maps close to the gene to be isolated and 'marching' along the chromosome by isolating overlapping DNA sequences (S. Artavanis-Tsakonas & Hogness, Stanford). The present rate of 15 kb per month may be accelerated by using vectors which can accommodate very large inserts of donor DNA.

Polytene chromosomes

The structure and function of polytene chromosomes remains a controversial field to which the recent studies, using fluorescent antibodies to localise RNA polymerases and other chromosomal proteins, have added a new dimension. Classical genetic studies indicate a strong correlation between genes and bands, but the analysis is more or less confined to unique essential genes and it is difficult to estimate what fraction of the genes escapes detection in these genetic tests. In contrast to the hypothesis that the boundary between bands and interbands in the giant chromosomes is defined by specific DNA sequences, a more dynamic model has been proposed on the basis of fluorescent antibody studies (E. Bautz, Heidelberg). This model assumes that puffs and interbands represent the actively transcribed fraction of the genome which varies with the stage of development and between tissues. The well-founded hypothesis that puffs are activated genes has obtained further support by the elegant demonstration of active units of transcription in Balbiani Ring 2 of *Chironomus* by the Miller spreading technique and electron microscopy (B. Daneholt, Karolinska Institute, Stockholm). It appears that there is only one active DNA sequence of 37 kb being transcribed in a chromosome which, on the basis of microspectrophotometry, contains as much as 470 kb.

Endocrinology

Insect endocrinology has also received a new impetus. A plant ecdysone, ponasterone A, has been found with about 50 times the activity of the insect hormone β -ecdysone. Its biological action seems to be identical to that of the natural moulting hormone. Because ponasterone A can be labelled to high specific activity by catalytic tritiation of an unsaturated precursor, stachysterone C, it can be used to identify putative ecdysone receptors, which so far has not been possible (M.-A. Yund, Berkeley). Data from

several laboratories indicate binding constants of the order of 10^8 to 10^9 M. A similar binding constant has been reported for a juvenile hormone receptor. Moulting hormones have now been discovered in the ovaries of several insects and intermediates in the biosynthesis of ecdysone are being isolated and characterised (J. Hoffmann, Strasbourg). The peaks of ecdysone concentration coincide with the time of secretion of the egg shell during oogenesis, and with the time of cuticle formation during embryogenesis. Vitellin, the major yolk protein, seems to be under hormonal control by juvenile hormone in most insects with the exception of mosquitoes and possibly other diptera, where ecdysone seems to take over this role. In locusts, vitellin is a glycolipoprotein of 550,000 daltons which is derived from a precursor, vitellogenin, that is synthesised in the fat body (G. Wyatt, Kingston, Ontario). *In vitro* studies indicate that initially two polypeptides of 265,000 and 250,000 daltons are synthesised which may represent products from two different genes. The labelled precursor can be chased progressively into a group of smaller polypeptides of 140,000 to 52,000 daltons. Vitellogenin synthesis is induced by juvenile hormone, secreted by the fat body and transported to the oocytes, which makes the system analogous to vitellogenin synthesis in vertebrates. Since methods are now available for the isolation of hormone-inducible genes and several ecdysone-inducible genes have already been isolated in *Drosophila* (Kemp & Hogness, Stanford), one can expect rapid progress in this area.

In the area of insect cell culture there has also been considerable progress. *Drosophila* cell lines have now been established from several mutant strains (H. P. Bernhard, Biozentrum Basel), and successful cell fusion has been announced (C. Wyss, ETH Zurich). Perhaps the most important recent contribution has been the establishment of a mutant cell line with the haploid chromosome number (G. Echallier, Paris).

Although there has been an extensive search for regulatory mutants, *Drosophila* geneticists have so far failed to provide conclusive evidence for the existence of regulatory elements controlling the activity of structural genes in development. It may be that our ideas have been too greatly influenced by models developed for prokaryotic systems. Interesting new results on genes controlling compartment formation in imaginal disks (T. Kornberg, University of California, Los Angeles; P. Lawrence, MRC Laboratory of Molecular Biology, Cambridge) and mutants interfering with sex de-

termination were presented (R. Nöthiger, Zürich). If such genes can be isolated by molecular cloning techniques, it may become possible in the future to unravel controlling mechanisms operating at these higher levels.

The workshop and the conference were among the best meetings that I have attended, especially in promoting the dialogue between scientists working in related fields, and our Greek hosts merit special thanks for providing an ideal setting which made these meetings so pleasant. □

Ice dynamics

from a Correspondent

Is man's effect on world climate likely to precipitate a catastrophic rise in world sea level as suggested by Mercer (*Nature* 271, 32; 1978) and is a major surge of the Antarctic ice sheet still considered possible by glaciologists? These and other questions were discussed during the recent International Glaciological Society's Symposium* on 'The dynamics of large ice masses' at Carleton University, Ottawa. Thanks to the associated 'Symposium on glacier beds: the ice-rock interface' organised under the National Research Council of Canada at the same location during the preceding week, there were many healthy exchanges between glacial geologists who had studied the effect of ice dynamics in the past, glaciologists studying present day glaciers and ice sheets and an increasing number of glaciological modellers. Most participants benefitted from attending both symposia, a happy juxtaposition of meetings since the glacier bed is the inadequately understood base of glacier dynamics in more ways than one.

It is apparent that while our understanding of the internal deformation of ice sheets is advancing along satisfactory lines, our knowledge of how much sliding takes place at the glacier bed, how much it is controlled by rock debris in basal ice, by basal water pressures and by pressure melting and regelation are still far from adequate. Until this is cleared up, modellers will lack guidance on how to treat the base of the ice sheets of Greenland and Antarctica. In this connection, long awaited reports on the debris-laden ice cores from the base of the ice sheets at Camp Century, Greenland and Byrd Station, Antarctica were of special interest.

A. J. Gow and W. Sheeby (US Army's Cold Region Research and Engineering Laboratory, Hanover, New Hampshire) and S. Epstein (Caltech) showed that the lowest 4.83 m of the core from Byrd Station contrasted with

* Held 21-25 August, 1978.

ice immediately above through its very low content of dissolved air, the presence of rock particles ranging from clay size through sand to pebbles and cobbles, and in its oxygen isotopic composition. The latter was around 4‰ lower in $\delta^{18}\text{O}$ values in the basal ice, and more variable than in the ice above. Thus when one allows for fractionation effects it seems that the basal ice that was formed by refreezing of the melt water was of fairly local origin, but it is not clear whether freezing took place in a single period or was episodic. Melt water was present beneath the ice when the base was reached in 1968.

Whereas debris weight percentages of up to 15.7% were present in the basal ice from Byrd Station, Susan Heron and C. C. Langway (Buffalo University) found an average debris concentration of only 0.24% in the bottom 15.7 m of the 1966 core from Camp Century. This contained over 300 alternating bands of clear and debris-laden ice. Although the basal ice temperature is now -13°C , they conclude that the debris was incorporated by freezing of subglacial water over a basal till. There was only one pebble in the Camp Century ice, the remaining fine debris being either distributed throughout the dirty ice layers or included in the form of pebble-sized aggregates of fine particles picked up from the basal till. In contrast to the Byrd core, the gas concentration in the basal ice at Camp Century was about half that at higher levels. The gas showed traces of methane, high CO_2 levels, low O_2 levels and enrichment of argon. It was suggested that although air may have been excluded during freezing, its composition indicated subsequent diffusion into the basal ice.

As with glacier beds, the main advances in our understanding of the dynamics of the Antarctic ice sheet are coming from field measurements often involving new techniques. Radio echo mapping from long range aircraft, satellite altimetry and Doppler position fixing as well as more conventional survey methods are providing much better knowledge of the form and flow of the polar ice sheets than seemed possible a few years ago. N. Young (Melbourne University, Australia) who took part in Soviet traverses over the east Antarctic ice sheet during the past two summers found that ice velocities measured by satellite Doppler techniques agreed with calculated balance velocities within an accuracy of 20% in a region of around one million km^2 between Mirny Station and Dome 'C'. Other reports indicate that areas feeding the Lambert and Shiraze Glaciers may not be in balance, while

evidence discussed by K. Rose of the Scott Polar Research Institute suggested that an ice stream feeding the Ross Ice Shelf from Byrd Land may have surged in the not too distant past. The general consensus appeared to be that while surges of individual glacier basins in Antarctica might occur, the idea of a single massive surge of 10 to 20% of the total ice mass of Antarctica as a cause of ice ages (Willson *Nature* 201, 147; 1964) was losing ground.

Laboratory studies on ice cores from Law Dome by D. S. Russell-Head and W. F. Budd (Antarctic Division, Department of Science, Melbourne) showed that flow (shear strain) rates in ice with strongly aligned C axes were typically three to five times faster than in randomly-oriented polycrystalline ice, whereas crystal size had little effect on flow rates. These results matched well with measurements of borehole inclination from the same locality. It is becoming clear that the variation of horizontal ice velocity with depth is more complex than expected and that the accuracy of age-depth relationships in ice cores based on ice flow models is limited by these factors.

The above did not, however, deter the modellers who use computer simulations to increase our understanding. Of these, a catastrophe model of the palaeoclimate by D. R. MacAyeal (University of Maine, Orono) that considered changes of insolation, sea level and isostatic depression of the glacier bed helped to explain the sudden transitions between ice-free and ice age conditions.

In addition to ice sheets, floating ice shelves and sea ice were discussed in half day sessions. Towed side scan sonar records from a ship obtained by O. Orheim of Norsk Polarinstitutt showed both basal scour of the sea bed by icebergs and a fascinating pattern of irregular melting at a depth of 100 to 200 m beneath straight cliffs of the ice front. Discussions of the mechanical and thermal stability of ice shelves and marine ice sheets indicate that before any sudden climatic warming would release the ice sheet of West Antarctica and affect sea level there would be a delay of some hundreds of years. After this, considerable progress in modelling of Arctic pack ice seemed relatively mundane, if no less important climatologically.

Overall, it is becoming clear that to advance from a few elementary but over-simplified concepts of glacier behaviour to a detailed understanding of the dynamics of ice sheets involves more complex investigations than was commonly realised a decade ago, but much progress is being made. This is significant since hydroelectric projects

and the development of Arctic oil involves judgement on glaciological matters that are of major importance to industry. □

New magnetic structures in the light rare earth metals

from S. B. Palmer

RECENT weeks have seen two significant developments that have cast new light on the magnetic behaviour of the rare earth elements, praseodymium and neodymium. At room temperature both elements are paramagnetic with localised magnetic moments produced by the electrons in the partially filled 4f shell. In the majority of the rare earth metals the exchange interaction is sufficiently strong to produce magnetic order at low temperatures although the oscillatory, long range nature of the exchange interactions results, in many cases, in complicated modulated magnetic structures. Each magnetic ion also experiences an electrostatic field with the symmetry of the crystal lattice: this crystal field interaction opposes the exchange by favouring a uniform arrangement of the moments. For both neodymium and praseodymium the crystal field and exchange energies are comparable, but in neodymium the exchange energy is sufficiently strong to produce magnetic ordering below 20 K.

The magnetic structure of neodymium has been a mystery since the initial neutron diffraction measurements of Moon *et al.* (*J. appl. Phys.* 33, 1041; 1964) carried out at Oak Ridge. The double hexagonal close packed (dhcp) structure has two separate sublattices, one with cubic and the other with hexagonal symmetry. Moon proposed that at 20 K the ions on the hexagonal sites ordered antiferromagnetically. These sites form planes lying perpendicular to the hexagonal axis and in a particular basal plane the magnetic moments, according to Moon *et al.*, are confined to the plane and oriented along the crystallographic *b* direction. The moments are sinusoidally modulated with the *q* vector of the modulation in an *a* direction, at 90° to the moments.

This model, however, was inadequate to explain all the features of both Moon *et al.*'s results and the more recent work of Lebech and Rainford (*J. Physique* 32, 370; 1971; *Proc. Int. Conf. Mag. Moscow* 3, 191; 1974.) A

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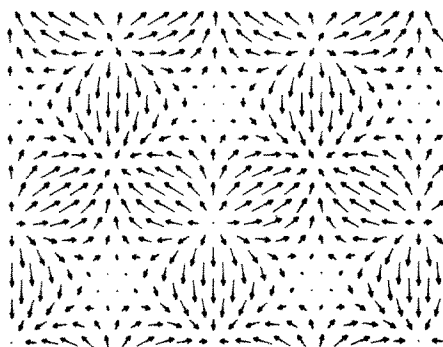


Fig. 1 Magnetic structure of neodymium for $\alpha=0^\circ$. The basal-plane components ($\uparrow$) of the spins in the A layers centred at the atomic positions of the undistorted lattice.

better fit to the experimental data could be obtained if it was assumed that the cooperative order on the hexagonal sites induced magnetic order on the cubic sites although the results were still far from satisfactory.

The report by Bak and Lebech (*Phys. Rev. Lett.* **40**, 800; 1978), of a magnetic structure that fits all the experimental results brings relief to many solid state physicists who have spent long hours attempting to unravel the neodymium puzzle. The novel magnetic structure of the hexagonal sites is shown for one basal plane in Fig. 1 where the magnetic moments are modulated in the three equivalent ' a ' directions simultaneously, hence the name of 'triple- q ' modulated magnetic structure. The work of Bak and Lebech in Denmark involved experimental proof that the magnetic phase change at 20 K was of second order, hence allowing Landau symmetry arguments to be used with renormalisation group theory to predict that the three different equivalent q vectors are present simultaneously rather than contained in separate domains as in the structure of Moon *et al.*

Associated with the magnetic structure shown in Fig. 1 are lattice distortions that are identical in form except for a phase difference of 90° . Initial estimates indicate that these distortions may well be of the order of a few percent and as such should be detectable by X-ray scattering studies.

Measurements of specific heat, elastic constants, magnetoresistance and neutron diffraction have all revealed a multitude of phase changes as a function of applied field in the temperature range 1-20 K. The extension of Bak and Lebech's model to high fields is eagerly awaited.

In contrast to neodymium, praseodymium is fascinating, not for the order it exhibits, but for the magnetic order it nearly exhibits. Experiments on single crystal praseodymium demonstrate that it remains paramagnetic down

to 30 mK: the exchange is about 90% of the critical value for magnetic ordering. In contrast polycrystalline praseodymium, highly strained because of anisotropic thermal contraction, orders antiferromagnetically at 25 K. The addition of 3% neodymium to single crystal praseodymium produces order below 6 K. Because of the large orbital angular momentum of the Pr ions, there is a powerful coupling between the lattice and spin systems. Jensen's analysis (*J. Phys. C* **9**, 111; 1976) of this magnetoelastic coupling led him to predict that, at 4.2 K, a relatively modest pressure of 1,000 bar applied along the crystallographic a direction would, by distorting the basal plane, change the delicate balance between exchange, crystal field and magnetoelastic energies and induce magnetic ordering. In a recent investigation of these predictions McEwen *et al.* (*Phys. Rev. Lett.* **41**, 343; 1978) have carried out (at the Institut Laue-Langevin, Grenoble) a series of careful neutron inelastic scattering studies of praseodymium as a function of uniaxial stress applied along the a axis. They found that as the stress was increased the

lowest energy magnetic excitation dramatically decreased, heralding the antiferromagnetic order which was established at 800 bar. The presence of the ordered phase was confirmed by the observation of elastic satellite reflections corresponding to a long-range magnetic structure. At 800 bar the Néel temperature was 7.4 ± 0.4 K. On removal of the stress the magnetic satellites were absent and the exciton dispersion curve was identical to that of previous workers and to the curve obtained before stressing took place. Both these results indicate that there was no residual stress left in the sample and the process was completely elastic.

The magnetic structure postulated by McEwen *et al.* is a basal plane modulated structure, identical to that of Pr - 3% Nd and reminiscent of the neodymium structure discussed above. Jensen's elegant theories, which have already led to quantitatively accurate explanations of neutron inelastic scattering studies of exciton phonon coupling, field dependence of elastic constants and magnetostriction, seem to have been vindicated again. □

Sun-weather effects

from Roger H. Olson

MOST of the papers presented at a recent conference on Solar-Terrestrial Influences on Weather and Climate* reflected the fact that Sun-weather research is still pretty much in the empirical stage. There is even disagreement on the details of many of the empirical findings. For many years the conventional wisdom has held that Sun-weather effects were stronger in winter than in summer, and stronger in the auroral zone than in low latitudes. However, this may be just a reflection of the fact that changes are more dramatic in high latitudes and in winter, and empirical studies tend to focus on the more dramatic effects. As far back as 1965, H. C. Willett of the Massachusetts Institute of Technology pointed out that, when properly normalised, the weather data show stronger responses to solar signals in summer and in low latitudes. Although there is still conflicting evidence, Willett's ideas received strong support from several investigators. There was also support for the idea that the X-ultraviolet portion of the solar spectrum may be more important than the particle emission, that is, solar proton events.

As for low energy solar particles, the so-called auroral particles, there was relatively little discussion of geomagnetic-weather effects. One possible reason is that in using geomagnetic data there is the danger that a small part of the geomagnetic signal could be caused by a weather disturbance. Although this danger is rather slight, many investigators prefer to use solar signals that are unmistakably solar, such as solar flares and plages, sunspots, and magnetic sector boundaries. One particular class of geomagnetic disturbances did receive special attention, however—the Forbush decrease events. In a Forbush decrease, the magnetic disturbance causes a decrease in cosmic ray flux throughout the whole depth of the atmosphere, including the surface layers. Thus, in seeking mechanisms to explain the various Sun-weather effects observed, the Forbush decrease is becoming more and more interesting.

One theme that ran through the conference was the importance of Sun-weather effects in weather forecasting. In 1977 M. F. Larsen and P. M. Kelley of Cornell University found that the accuracy of US weather forecasts took a dip one day after solar sector boundary crossings. Following their lead, R. Reiter (Institute for Atmospheric Environmental Research of the Fraunhofer Society, FRG) reported that German forecasts suffered a

* The first Ohio State University Sun-Weather Conference was held on the campus of the University from 24-28 July, 1978. The conference was organised and chaired by T. A. Seliga, Ohio State University, and B. M. McCormac, Lockheed Palo Alto Research Laboratory. It is hoped that this type of conference can be repeated every two to three years.

similar fate. J. W. King (Appleton Laboratory, UK) reported similar findings for British forecasts. All these results cover relatively short time spans and limited geographical areas, and thus should be treated as tentative, but highly suggestive.

There was a healthy degree of skepticism expressed by several speakers. W. O. Roberts (Aspen Institute for Humanistic Studies, Boulder) stressed that, in spite of the virtual certainty that Sun-weather relationships exist, we desperately need rigorous theoretical studies and models to explain them. J. Namias (Scripps Institution of Oceanography, La Jolla) chose to focus on one meteorological parameter, namely drought. He stressed the importance of understanding the full implications of the physical causes of droughts in attempting to correlate drought with solar activity. Interestingly enough, although drought is typically associated with anticyclones, he felt that the best place to look for Sun-weather influences is in cyclonic activity. His attitude is perhaps typical of many highly skilled and experienced meteorologists—namely that Sun-weather research, in spite of its long and frustrating history, deserves careful scrutiny. The chief skeptic by far, was A. B. Pittock, temporarily of the University of Arizona. He cast strong doubts on many published reports of correlations between weather and the various solar cycles (such as 27 days, 11 years and 22 years). The 27-day cycle was also attacked by others, who pointed out that it is difficult to distinguish between 27-day solar and lunar effects. Pittock had little quarrel with some of the short-term correlations which have been reported recently.

As regards mechanisms, the cupboard is not entirely bare. Perhaps the most popular was the thunderstorm mechanism, discussed by R. Markson (Massachusetts Institute of Technology) (see *Nature* 273, 103; 1978) and others. He regards the thunderstorm, and its associated high resistivity to vertical electrical currents as a valve controlling the whole electric circuit of the atmosphere. Solar flares, and possibly Forbush decreases, can modify the resistivity above thunderstorms, leading to profound changes in the global circuit. This could feed back into the physics of the clouds, affecting precipitation patterns and affecting gross features of the atmospheric energy balance. Empirical evidence supporting this idea was furnished by Markson himself, by R. Reiter, and

by M. Lethbridge (Pennsylvania State University). They all presented evidence that thunderstorm frequency is related to solar signals such as flares and solar sector boundaries.

Ozone was also mentioned as a possible trigger mechanism. M. B. Schlesinger (Oregon State University) suggested that an initial ozone increase, caused by solar activity, could lead to circulation changes, which in turn would lead to advective changes in ozone concentration, this leading to a positive feed-back. Many others agreed that some sort of ozone mechanism might be useful. Another possible feed-back mechanism was suggested by E. C. Kindle (Old Dominion University, Norfolk, Virginia) and also elaborated by others. This involves the Charney-Drazin effect, which operates on about a 10-day time scale, a typical time scale for some reported Sun-weather effects. If the speed of the middle latitude westerlies is decreased, this allows greater penetration of tropospheric wave energy up into the stratosphere. Thus if the troposphere is subjected to an influence (possibly a solar influence) that causes it to lose kinetic energy, the feed-back mechanism

will cause it to lose even more energy through dissipation to the stratosphere.

The symposium ended with a number of resolutions. One involved a request to international scientific organisations, such as ICSU, to place the Sun-weather problem high on the list of future projects. Another was to decry the tendency for old, reliable data sets to be threatened with extinction, due to retirement of key personnel or lack of funding. Examples of data sets which are either threatened or actually ended include the Zurich sunspot observations, the Ottawa centimetric radio observations, the Greenwich daily sunspot drawings, and the McMath daily plage observations. These have been of great value both to research workers and to those engaged in operational solar-terrestrial problems. As an example of the latter, there were reports given by two commercial weather forecasters who use solar data in making their forecasts. Although it may be regarded as premature, even by some enthusiasts, to use solar flares, for instance, in routine weather forecasting, the fact that it is being done underscores the need for a serious study of the problem.

Therapeutic agents for sickle cell disease

from M. F. Perutz, J. Rosa and A. Schechter

At a recent workshop in Paris* the molecular and cellular bases of sickle cell disease and possible new approaches to its therapy were discussed.

M. F. Perutz (MRC Laboratory of Molecular Biology, Cambridge) reviewed X-ray diffraction and electron microscope studies of the gels of deoxyhaemoglobin A that precipitate in the red cells of patients with sickle cell anaemia. These gels consist of fibres of 20–22 nm diameter, each made up of several filaments of 6 nm diameter in which single haemoglobin molecules are strung together like pearls. The structure of the contacts between successive molecules in these filaments is known in atomic detail from X-ray analysis of crystalline deoxyhaemoglobin A in which the molecules are strung together in the same way. Sickling is probably caused, first, by the pairwise aggregation of such filaments, as in crystals of deoxyhaemoglobin S (deoxyHb S), where the valines of one filament touch nonpolar side chains between helices E and F β in the neighbouring filament of the pair, and then by the aggregation of several such pairs to form a fibre. Dykes *et al.* proposed

that each fibre is a rope made up of 14 filaments (*Nature* 272, 506; 1978); A. Klug suggested that this number may arise from the winding of six pairs of filaments around one central pair.

W. Love (Baltimore) reported that the resolution of the X-ray analysis of single crystals of deoxyhaemoglobin S had been raised from 5.0 to 3.0 Å, good enough to distinguish individual residues. The new map confirms that the structure of deoxyHb S is the same as that of deoxyHb A, except that the substitution of valine for glutamate 6 β may have forced the first turn of helix A to a conformation different from that in deoxyhaemoglobin A. B. Magdoff-Fairchild (New York) reported her exciting discovery that oriented, paracrystalline preparations of sickled cells or deoxyhaemoglobin A can turn into bundles of crystals isomorphous with those analysed by Love and his colleagues. The intensity distribution in the X-ray diffraction patterns of these crystalline bundles is similar to that of oriented gels, suggesting similar molecular arrangements. This raises hopes that by combining information from crystals and gels we may be able to find out more about the structure of the contacts between molecules in the

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* Held on 19–21 July and sponsored by INSERM and the US National Institutes of Health

gels than has been possible by studies of gels alone.

At constant temperature solutions of deoxyHb S remain liquid for long periods until they suddenly gel. The delay is exactly reproducible and is inversely proportional to high powers of the temperature and haemoglobin concentration (*Proc. natn. Acad. Sci. U.S.A.* **71**, 4864; 1974). W. Eaton (Bethesda, Maryland) proposed to use this phenomenon for testing anti-sickling drugs. To make the disease significantly less severe, a drug would have to increase the delay 10–100-fold, equivalent to a 1.06–1.17-fold increase in the solubility of deoxyHb S or a 1.5–4-fold decrease in intracellular Hb concentration or a 4–7-fold increase in oxygen affinity. These are stiff requirements. E. R. Heuvels and M. Rosemeyer measured the antigelling properties of Hb A, F and A₂ when added to Hb S, and found that the antigelling effect rises in that order. This suggests that activation of the gene for Hb A₂ would have a more potent antisickling effect than activation of the gene for Hb F.

Cell studies

Studies of whole normal and sickled cells were reviewed next. J. Hoffman (New Haven) discussed the distribution of diffusible ions and control of cell volume in normal erythrocytes, and the use of fluorescent dyes to study the intracellular milieu. Such studies are very relevant in view of the steep dependence of the gelling delay on haemoglobin concentration. M. Mohandas and N. Bessis (Paris) used anisotropy of laser light scattering to measure the deformability of red cells sheared between the concentric cylinders of a viscometer. This method could be used to measure the degree by which antisickling agents diminish the stiffening of deoxygenated sickle cells.

The physiology of the sickle erythrocyte is more complex than that of Hb A solutions. Recent technical advances are now allowing their oxygen equilibria to be measured. R. Bookchin (New York) found that the Bohr effect of sickle cells is steeper than that of normal ones and that 2,3-diphosphoglycerate, the intracellular oxygen regulator, increases gelation merely by lowering the oxygen affinity and not, as had been reported, by making deoxyHb A less soluble. R. Benesch and R. E. Benesch (New York) measured the oxygen affinity of Hb A as a function of haemoglobin concentration and found a discontinuity at

the point of gelation corresponding to the minimum gelling concentration. The concentration at which this discontinuity occurs could be used as a measure of the antigelling properties of drugs. If any drug inhibited sickling without raising the oxygen affinity of solutions which are too dilute to gel, then this would prove that it acted on the intermolecular contacts.

Antigelling and antisickling agents

Compounds being tested *in vitro* as antigelling or antisickling agents were discussed next. These may act by more than one mechanism, but can be classified according to their primary effects. The first class consists of modifiers of Hb A which raise the oxygen affinity. The prototype of such reagents was KNCO which proved too toxic for oral therapy. According to L. Kraus carbamylphosphate reacts with haemoglobin in the same way as cyanate, but the unreacted fraction may be less toxic as a result of enzymatic degradation. Imidoesters, both bifunctional dimethyladipimidate (B. Lubin, Oakland, California) and monofunctional methylacetimidate (T. Gabuzda, Philadelphia) decrease sickling by raising the oxygen affinity; so does cystamine which reacts with cysteines 93β (Y. Beuzard & J. Rosa, Paris). Among disulphide reagents cystine dimethyl-ester reacts with the same groups, increases the oxygen affinity and has antisickling properties demonstrable in single erythrocytes (D. L. Currell, Rome). The toxic nitrogen mustards react with histidine 2β which is one of the diphosphoglycerate binding groups. (E. Roth, New York).

The second class consists of modifiers of intermolecular contacts in deoxyHb S fibres. J. Manning (New York) has found that glyceraldehyde inhibits sickling, probably by reaction with a specific lysine not yet determined. It also acts on the membrane. Other compounds in this class interact non-covalently, such as the alkylureas studied by R. Nagel's group (New York) which are more potent inhibitors than urea itself. A. Schechter (Bethesda) has sought stereospecific amino acids or peptides that inhibit gelation and found that aromatic amino acids or peptides containing aromatic amino acids, but not the sickling peptide (residues 1–16β), have antigelling properties. C. F. Poyart (Paris) found that methane, ethane, propane and butane increased the oxygen affinity by 10–15%, propylene by 25% and butane by 50%; these agents may also act on the intermolecular contacts. A. Cerami (New York) reported experiments with n-carboxyaspirindimethylester which reacts with an as yet undetermined group.

The final class of compounds dis-

cussed were modifiers of the whole erythrocyte. G. Brewer (Ann Arbor) described clinical and laboratory studies of Zn acetate as an antisickling drug and C. Natta (New York) discussed DBA(3,4-dihydro-2,2-dimethyl-2H-1-benzopyran-6-butyric acid).

Many of these compounds are too toxic for oral use and would have to be administered extracorporeally, by removing the red cells from the circulation, reacting them with the drugs, and putting them back again. S. Balcerzak (Columbus) has tested extracorporeal administration of cyanate to erythrocytes of dogs and two baboons and found no toxic effects in periods of up to one year. D. Diederich (Kansas) tried extracorporeal cyanate treatment on about 10 sickle cell patients and found a reduction in crises during treatment, as compared to before and after.

According to W. Rosse's (Durham, North Carolina) review of the American clinical trials of orally administered urea, alkali and cyanate, none of these reduced the severity or frequency of sickle cell crises, but the trials helped to establish reliable criteria for evaluating new therapies. At a final round table conference clinicians from West Africa and the USA dwelt on the great variability of the disease, and the absence of reliable information concerning its mortality, morbidity and clinical manifestations, especially in Africa and the Middle East. They were also concerned about the ethical problems of trying anti-sickling drugs on patients. They would not try them on children and felt that even adults might be better helped by conservative treatments. □



A hundred years ago

FROM the 16th to the 18th of August the city of Cartago in Costa Rica, in Central America, was visited by five distinct shocks of earthquake. No particular damage was caused. What is to be noticed in the meteorological result: that the weather was changed, being attended by heavy showers that flooded the rivers. In all earthquake countries it is believed the weather is affected by such phenomena, and it is desirable that observations should be recorded, showing the influence of earthquakes on weather. It has been alleged that the Comrie earthquakes in Perthshire came on after rains and floods of the river.

From *Nature* 3 October, 18, 600; 1878.

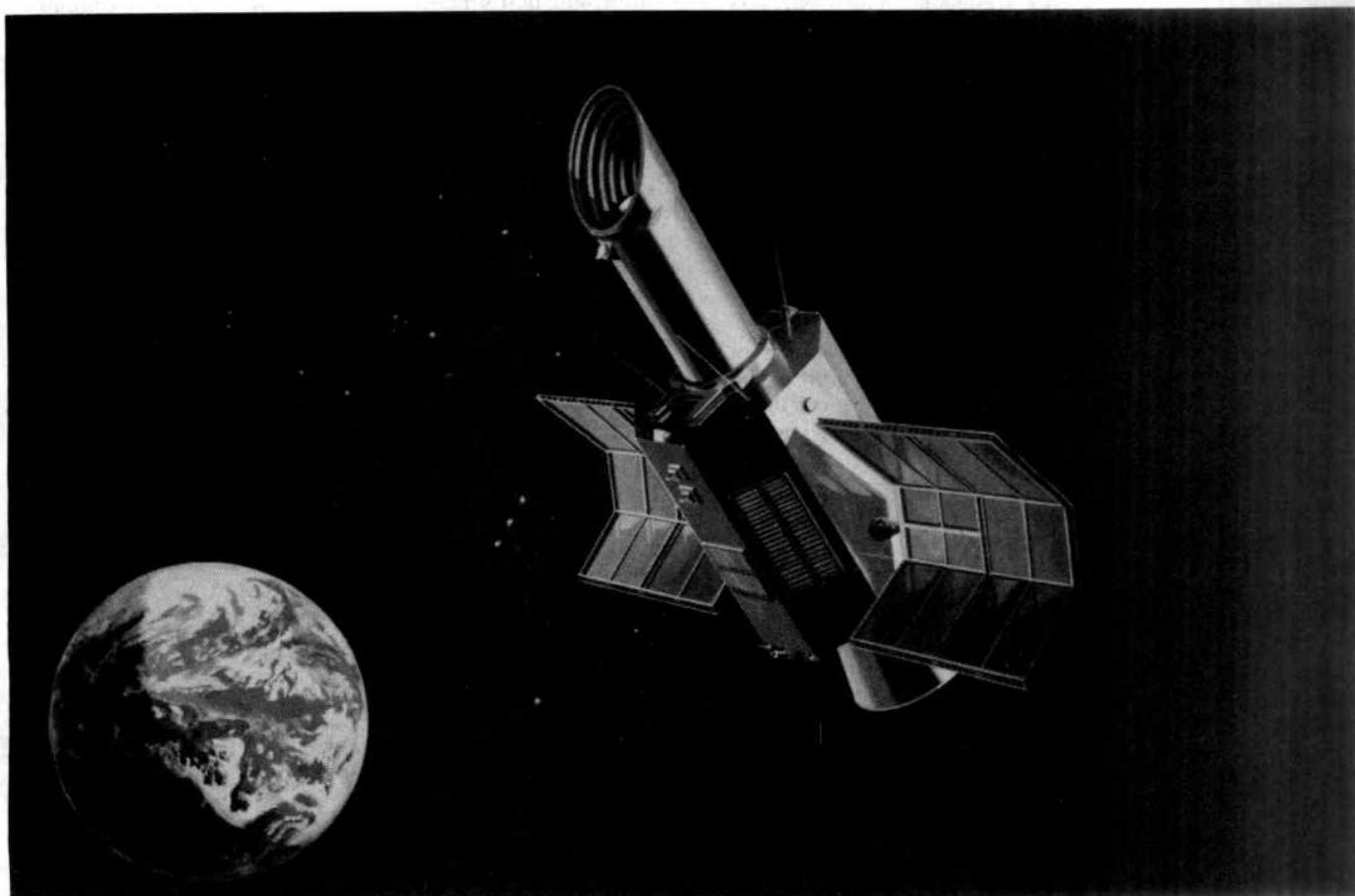
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The International Ultraviolet Explorer (IUE)

The following eight papers deal with the International Ultraviolet Explorer (IUE), a joint project of the National Aeronautics and Space Administration (NASA), the European Space Agency (ESA) and the Science Research Council (SRC). The first paper describes the overall project and concept. The second deals with its performance in orbit as assessed during the commissioning phase following the successful launch of 26 January 1978. In addition to engineering switch-on, sub-system tests, optimisation and calibrations, that commissioning phase included astronomical observations of a set of high-priority targets as an insurance against premature failure of the system. What is 'high priority' is always a matter of judgement, and in this case that judgement was made by an international science commissioning team drawn mainly from the project staff of the three agencies but also including scientists from the general community. The results of those high-priority observations and their subsequent analysis are presented in the final six papers of this series. It should be stressed that these observations were made immediately after switch-on, that is before the system had been fully optimised or calibrated, and that the data were reduced in the very earliest phases of the image processing system. Nevertheless they clearly indicate the potential of IUE and provide a range of new and exciting data in many areas of astronomy.

In addition to the authors listed on the following papers, many people have made essential contributions to this project in all its stages, from conception and approval through hardware production and launch to the recent commissioning and operational phases. To all those people, in the government agencies, research institutes, universities and industry of many countries, the authors extend their thanks and gratitude. These papers were submitted on 9 August 1978.

R. Wilson



The IUE spacecraft and instrumentation

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The International Ultraviolet Explorer satellite operates as an astronomical observatory to provide both high and low resolution UV spectra of sources other than the Sun. The spacecraft, its instrumentation and the ground control system are described.

THE International Ultraviolet Explorer (IUE) was launched successfully on 26 January 1978, from Cape Canaveral, Florida. The satellite was developed to provide a general facility for observing ultraviolet (UV) spectra of astronomical sources over the wavelength range from about 1,150 Å to 3,200 Å. The project has been a joint undertaking in which the US National Aeronautics and Space Administration (NASA) provided the spacecraft, the optical and mechanical components of the scientific instrument, the US ground observatory and spacecraft control software; the UK Science Research Council (SRC) in collaboration with University College London (UCL) provided the television cameras used to record the spectroscopic data; and the European Space Agency (ESA) provided the solar arrays and European ground observatory. The image processing software was developed jointly by NASA and the SRC's Appleton Laboratory. The satellite has been placed in geosynchronous orbit over the Atlantic Ocean and is operated for 16 h each day, for NASA sponsored observers, from the US ground observatory located at the Goddard Space Flight Center (GSFC) near Washington, D.C., and for the remaining 8 h by ESA, for ESA and UK sponsored observers, from the European ground observatory located near Madrid.

Here the spacecraft and its instrumentation are described. In the following series of papers IUE's performance in orbit will be discussed¹, and the initial results from early observations taken during the satellite checkout and commissioning period reviewed²⁻⁷.

Design concepts

It was decided that a high spectral resolution of the order of 0.2 Å would be required to study the atmospheric characteristics of bright stars and planets and that a lower resolution of about 6 Å should be available to obtain information on faint sources. In addition, two other requirements were fundamental in determining the design of the scientific instrument and of the spacecraft to support it: first, the instrument should be able to record simultaneous data over large spectral ranges and, second, the spacecraft should be able to take maximum advantage of the observer's real-time judgement concerning the quality and content of his data.

The first suggests a spectrograph using an imaging system, such as a television camera, capable of integrating the spectrum. A spectrograph with echelle optics gives a compact spectral

format with high dispersion and, since the echelle image consists of many adjacent spectral orders displayed in a closely spaced array, it makes efficient use of the field of view of a television camera. To achieve adequate resolution, the total spectrum must be split into two ranges, from 1,150 Å to 1,950 Å and from 1,900 Å to 3,200 Å, and so two cameras are required to record the full spectral range. The observing efficiency is hardly affected by this split, as two exposures are usually required to expose, optimally, both the long and short wavelength spectra.

The second requires good communication between the spacecraft and the observer so a geosynchronous orbit was chosen. The IUE orbit allows continuous real-time control from the NASA control centre and at least 10 h each day from the ESA control centre. The arrangement is fundamentally different from low altitude orbiting observatories which have to acquire data while remote from ground control. In the case of IUE, control and performance monitoring are exercised continuously. The telescope field is displayed to the observer, who identifies his target star and directs the course of the observation in real-time. The 'IUE observatory', thus comprises the ground control centre, where the astronomer views his television monitors, and the optical and electronic instrumentation located in synchronous orbit.

A synchronous orbit has other advantages. Because the Earth subtends an angle of only 17° the unconstrained area of sky is much greater than for low orbits and, as its movement along the ecliptic is at the diurnal rate, the occurrence of occultations is infrequent. Consequently, over large portions of the sky, neither

Table 1 Spacecraft characteristics

Characteristic	Description
Spacecraft mass	312 kg
Scientific instrument mass	122 kg
Apogee motor mass	237 kg
Launch vehicle adaptor mass	29 kg
Total launch mass	700 kg
Planned mission life	3-5 yr
Power required (Spacecraft and scientific instrument)	210 W average
Array capability (beginning of life)	424 W to 238 W depending on attitude
Launch vehicle	Delta 2914
Stabilisation and control	
Transfer orbit	Spin stabilised
Mission orbit	3-axis stabilised with better than 1 arc s control
Orbit	Elliptical geosynchronous
Inclination	28.6°
Apogee	45,887 km
Perigee	25,669 km
Eccentricity	0.24

long exposures nor the observation of variable phenomena need be periodically interrupted. A penalty is that in synchronous orbit observations are normally made in full sunlight so the telescope must be baffled to ensure adequate rejection of stray sunlight and earthlight. Fortunately, sunlight scattered from the Earth's atmosphere contains very little UV energy and so the limiting magnitude of the spectrograph is not appreciably affected by earthlight. The offset guiding system can be influenced, however, and relatively bright guide stars are needed near the Earth's limb.

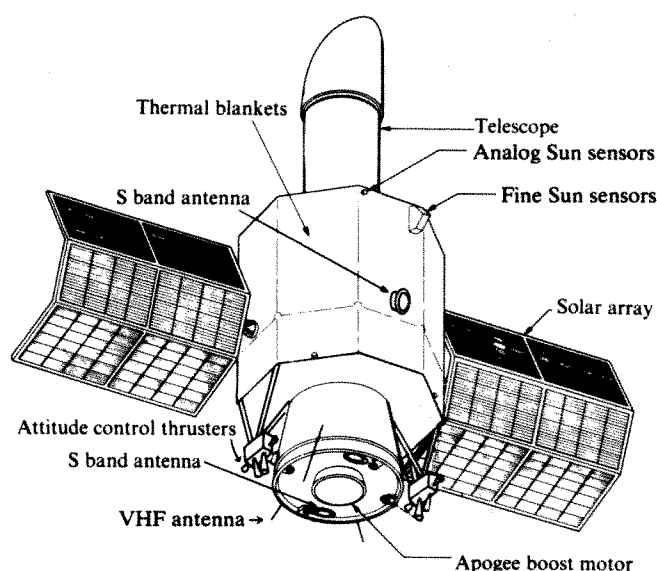


Fig. 1 IUE spacecraft.

General description of spacecraft

The general arrangement of the IUE spacecraft is shown in Fig. 1. The main body is octagonal in shape, the telescope protrudes from one end and the solar array extends from two opposite sides; in orbit the spacecraft is rotated around the telescope axis to keep the arrays nearly normal to the Sun. The apogee boost motor used to insert the spacecraft into synchronous orbit and the hydrazine auxiliary propulsion system used during the transfer orbit and for manoeuvres throughout the mission are both located in the lower cone assembly.

The telescope and spectrograph are rigidly connected together to form the scientific instrument to which the inertial reference assembly is directly attached to minimise relative displacement. An off-set star tracker within the scientific instrument provides the inertial reference assembly with a reference signal to enable drifts to be corrected. With this arrangement the pointing accuracy of the telescope is a fraction of an arc s.

Other spacecraft systems are described in later sections of this article and the spacecraft characteristics summarised in Table 1.

The scientific instrument

The scientific instrumentation consists of the telescope, the fine error sensors, the echelle spectrographs and the cameras. Each of these units will be discussed in turn.

Telescope and fine error sensor (FES): the telescope has an 0.45 m $f/15$ Cassegrain configuration with a Ritchey-Chrétien figure, giving uniform image shape across the 16 arc min field of view. The characteristics of the optics are summarised in Table 2. Telescope materials were chosen for their light weight, moderate cost, and thermal and optical properties. Beryllium was selected for the primary mirror, and fused silica for the secondary mirror. The telescope tube is aluminium, wrapped in aluminised Mylar thermal insulation; this thermal design produces a nearly constant focus over a wide range of solar aspect angles, and after initial focussing in orbit by movement of the

secondary mirror, no further refocussing should be required. Small corrections to the focus can be made using heaters behind the primary and secondary mirrors.

The forward sunshade is cut at a 43° angle with respect to the optical axis so that the telescope may be pointed anywhere in the sky more than 43° from the Sun. (The 43° angle was selected to permit observations of Venus when it is at greatest elongation.) The forward rim of the sunshade has an additional diffraction edge so that sunlight must be triply diffracted before it can enter the telescope tube. The small amount of diffracted sunlight that enters the telescope tube is further reduced by light baffles inside the tube. The most troublesome source of stray light is the Earth, which may appear at any angle with respect to the telescope axis. The baffles inside the sunshade are angled so that the light is preferentially scattered back out of the sunshade and all off-axis light must suffer at least two scatterings before it can reach a telescope mirror. With this design, stray light from the Earth or the Moon will be of negligible intensity as long as the primary mirror is not directly illuminated, but this can occur if the Earth's limb or the Moon is less than 25° from the optical axis.

Behind the primary mirror there is a shutter which closes automatically if sunlight falls inside the telescope tube. Behind the shutter, the spectrograph entrance apertures are mounted in the focal plane. These apertures are holes drilled in a mirror mounted at 45° to the telescope optical axis and which reflects the telescope field surrounding the apertures into duplicated FES, each of which serves the dual purpose of offset star tracker and field camera. Either is able to scan, in 8 arc s steps, the 16 arc min telescope field, an image of which can be transmitted to the ground for identification by the observer. The FES then measures the coordinates of the target star with respect to the spectrograph entrance apertures so that the spacecraft may be manoeuvred to centre the object in the selected entrance aperture. The FES is then offset to any other star in the field to generate error signals to hold the telescope on target. The limiting magnitude of the FES depends on its mode of operation, but it can track stars as faint as 14th visual mag. Thus any object, regardless of its brightness or nature, may be observed provided its position is known with respect to a nearby guide star.

Echelle spectrographs: behind the 45° aperture plate there are separate short and long wavelength echelle spectrographs, covering the spectral ranges 1,150–1,950 Å and 1,900–3,200 Å respectively. Each spectrograph has a pair of entrance apertures consisting of a hole 3 arc s in diameter and an oval slot 10×20 arc s. Both the larger slots can be closed simultaneously by a shutter, but the smaller holes always remain open. Any particular aperture can be selected by pointing the telescope so that the target image lies in that aperture. The arrangement of the optical components for the short wavelength spectrograph is shown in Fig. 2. Light from the target passes through the aperture plate to the collimator mirror, which is an off-axis paraboloid. The collimated beam is incident on the echelle grating and then diffracted to the spherical grating which acts

Fig. 2 Short wavelength spectrograph.

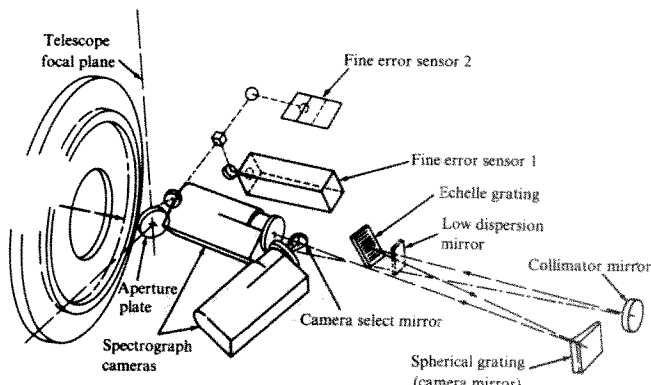


Table 2 IUE telescope parameters

Configuration	Cassegrain
Figure	Ritchey-Chrétien
Clear aperture	0.45 m
Central obscuration (baffled)	0.22 m
Primary focal length	1.25 m
Effective focal length	6.75 m
Focal ratio	15
Mirror separation	1.027 m
Back focal distance	0.175 m
Plate scale	30.6 arc s mm ⁻¹
Image quality	3 arc s
Field of view	16 arc min

both as an order separator and as a focusing element to image the entrance aperture on to the camera. By command, a camera-select mirror may be made to interrupt the beam and divert the image to a redundant camera. Similarly, a plane mirror (labelled the low dispersion mirror in Fig. 2) may be inserted in front of the echelle grating, resulting in a low dispersion spectrum produced by the spherical grating alone. The optical design of the long wavelength spectrograph is the same as the short wavelength spectrograph, except that two plane mirrors inclined at 45° are located behind the long wavelength entrance apertures to direct the incoming light beam to the long wavelength spectrograph and so separate its elements from those of the short wavelength instrument. In the long wavelength spectrograph the spherical grating could produce a second-order spectrum (from ~1,000 to 1,600 Å, which would overlay the 1,900–3,000 Å first-order spectral range), but this is suppressed by a silicon oxide coating on the 45° and collimator mirrors. The optical parameters of the two spectrographs are summarised in Table 3.

The spectrograph section of the scientific instrument also contains a number of light sources. The first of these is a platinum–neon hollow cathode lamp⁸ which is used as a wavelength standard. The lamp is mounted behind the primary mirror and illuminates the spectrograph entrance apertures by a mirror on the back of the Sun shutter so that a platinum spectrum can be recorded to calibrate wavelength as a function of position in the spectrograph image. Tungsten filament and mercury lamps are mounted in front of the cameras in each spectrograph to flood the camera faceplate with light. The former are used in the camera preparation procedure, described below; the latter⁹ can illuminate the cameras with predominantly 2,537 Å radiation, and are used to produce sets of flat-field images at different exposure times in order to derive intensity transfer functions for each picture element (pixel) of the camera format.

The spectrographs are fixed focus and, to minimise thermal effects, their temperature is closely controlled and the optics are mounted using tubes of graphite fibre-reinforced epoxy selected for its low coefficient of thermal expansion.

Spectrograph cameras: in each spectrograph a television camera integrates the two-dimensional echelle spectrum during an exposure and converts the image into video signals suitable for transmission to the ground. There are prime and redundant

cameras in both long and short wavelength spectrographs, all of nominally identical construction. Each camera head contains the electro-optical detector components and some of the electronics, such as the video head-amplifier, which must be close to the television camera tube. The remaining electronics are mounted in the spacecraft.

The basic detector is a UV-to-visible image converter (UVC) coupled to a secondary electron conduction (SEC) television camera which is sensitive to visible light only (Fig. 3). The entrance window of the UVC is magnesium fluoride, transparent to wavelengths above 1,150 Å, and on its inside face is a semi-transparent caesium–tellurium photocathode¹⁰ with a quantum efficiency of 10–15% in the UV but less than 0.01% in the visible. Within the UVC, a high electric field (5 kV across a 1.3 mm gap) accelerates photoelectrons onto a phosphor screen where each produces about 60 photons whose energy peaks in the blue. The screen efficiency is improved by a surface layer of aluminium which reflects backward-emitted photons through

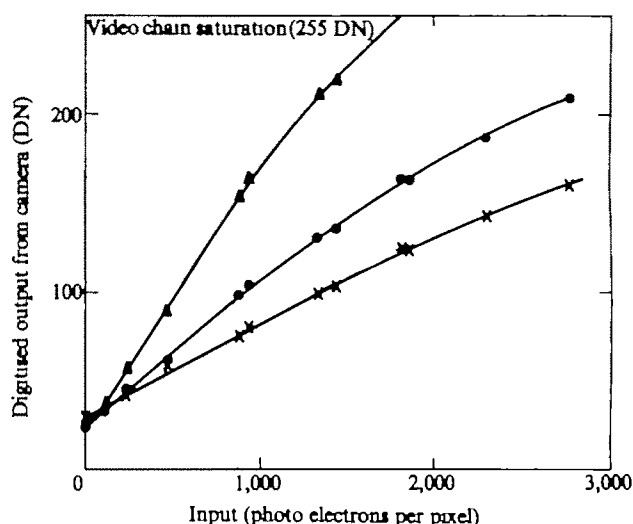


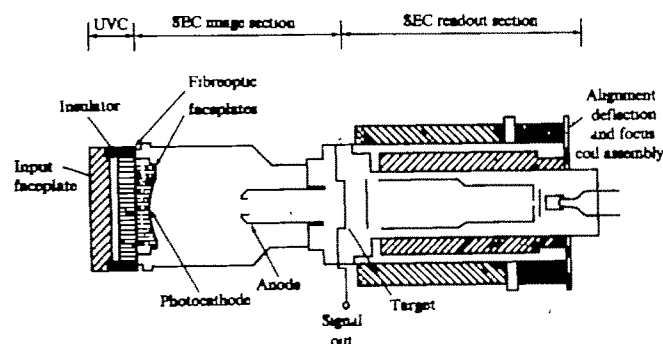
Fig. 4 Typical camera intensity transfer function for image centre (●) and positions of maximum (▲) and minimum sensitivity (×)

the output window; this layer also minimises optical feedback. A matt black coating on the input side of the aluminium reduces halation by suppressing reflection of UV photons which penetrate the photocathode.

The blue image from the UVC is transferred by fibre-optic coupling to the bi-alkali photocathode of the SEC tube. Incorporated in the fibre-optic coupling is a square array of fiducial marks to provide a geometrical reference for the spectral image. The photoelectrons, generated at the photocathode, are accelerated through the electrostatically-focused image section onto the SEC target which consists of a low density porous layer of potassium chloride about 10 μm thick, supported by an aluminium oxide membrane about 50 nm thick, and backed by a thin aluminium signal plate. Some fraction of the energy of each photoelectron is expended in producing several secondary electrons in the potassium chloride which are swept towards the signal plate by a 12V bias across the target leaving an amplified positive charge-image stored on the target, (the process of secondary electron conduction from which the SEC tube¹¹ is named). The gain depends on the accelerating voltage and is about 50 at the highest voltage setting of around 6.1 kV, because of the highly insulating nature of the target an image can be accumulated over many hours and there is no detectable reciprocity failure.

The image is read on completion of exposure. The readout section of the camera tube is similar to that of a standard magnetically-focused vidicon although its operation is somewhat different in that the beam is deflected digitally to scan a raster of 768 × 768 pixels in 37-μm steps across the image. At each pixel, the read beam is pulsed on and discharges the target,

Fig. 3 Camera showing UV to visible image converter and SEC tube



giving rise to an output video pulse corresponding to the positive charge on the target at that pixel. The video signal is digitised into one of 256 discrete levels (DN units) by an 8 bit A/D converter, and these data are telemetered in real-time.

Table 3 IUE spectrograph optics

Optical element	Short wavelength spectrograph	Long wavelength spectrograph
Wavelength range	1,150 to 1,950 Å	1,900 to 3,200 Å
Offset mirrors	None	Two at 45°
Collimator radius	1.89 m	1.89 m
Low dispersion mirror	Plane	Plane
Echelle grating		
frequency	101.9 mm ⁻¹	63.2 mm ⁻¹
blaze angle	45.5°	48.1°
off-normal angle	10.2°	10.2°
Spherical grating		
frequency	313.0 mm ⁻¹	200.0 mm ⁻¹
radius	1.37 m	1.37 m
Camera select mirror	Plane	Plane
High dispersion resolving power*	1.2 × 10 ⁴	1.3 × 10 ⁴
Low dispersion resolution*	6 Å	8 Å

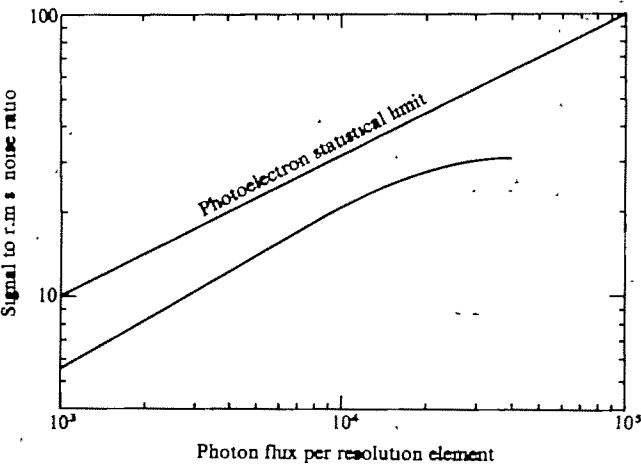
* Based on a predicted point spread function for the entire system (guidance plus optics plus detector) of 120 μm full width at half maximum. Actual resolutions achieved in orbit are discussed in the following paper¹

The cameras are operated by a series of commands issued by the ground station operator through keyboard procedures. Each operational mode has a number of parameters which may be selected by the guest astronomer. The normal image sequence is: PREPARE-EXPOSE-READ. A PREPARE operation is carried out before each EXPOSE to erase all trace of previous images from the target and to provide a reproducible low-noise baseline on which the new image will be superimposed. It consists of preprogrammed sequences of over-exposures to the tungsten floodlamps followed by READ scans. In the EXPOSE mode the UVC and SEC image section high voltages are on (the readout section of the SEC tube is off) and an image may be integrated on the SEC target. Exposure times may be controlled from the ground or by the on-board computer. In the READ mode the image section and the UVC are off and the stored charge image is read from the SEC target as described above.

Camera performance

The response of a camera to UV photons is nonlinear at high signal levels, owing to saturation effects in the storage of charge in the SEC target. The relation between the number of input photoelectrons and the output from the video chain is known as the intensity transfer function (ITF) (Fig. 4). As can be seen, the ITF varies across the image and individual ITF corrections are applied to each of about half a million pixels. The photometric linearisation of the data is an important element of data reduction¹².

Fig. 5 Typical signal-to-r.m.s. noise ratio per resolution element (3 × 3 pixels) as a function of the input to the camera at 2,537 Å.



The ultimate accuracy with which a spectral feature can be measured is set by the quantum statistics of the signal photons incident on the detector but the quantum efficiency of the photocathode reduces this accuracy and it is further degraded by extraneous noise sources which depend on such parameters as SEC target gain, signal level, method of preparation of the target, wavelength and the camera noise level. A typical signal-to-noise characteristic achieved in orbit after photometric correction is shown in Fig. 5.

The spatial resolution of the camera is determined by the combined effects of the UVC and SEC tubes. A typical modulation transfer function (MTF) is shown in Fig. 6; the very rapid drop in MTF at low spatial frequencies is a result of halation.

The dark currents in the UVC and SEC are mainly thermionic in origin and result in a typical background signal of 0.5–1.5 DN per hour of exposure. Images resulting from long exposures usually show a number of small bright spots which vary in time and position; these are probably the result of radioactive disintegrations in the phosphor. Bright spots and streaks can also be caused by interaction of cosmic rays with the input window of the UVC. Aluminium shields not less than 4 mm in thickness surround each camera to provide substantial protection against radiation from the Earth's trapped electron belts which would otherwise produce an unacceptably high background.

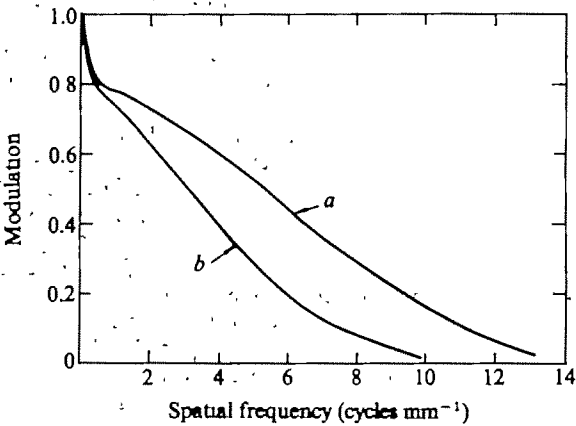


Fig. 6 Typical camera modulation transfer function for a, centre of image and b, at half radius (position of worst response).

Geometric distortions produced in the SEC tube amount typically to about 2% of the radial distance and consist of 'pin cushion' distortion produced by the electrostatically-focused image stage, 'S' distortion produced in the magnetically-focused read-out stage, small scale changes due to approximations used in the generation of the dynamic refocus waveform and small intensity-dependent image shifts in the read-out section of the SEC caused by 'beam-pulling' effects.

Spacecraft systems

The major sub-systems necessary to support operation of the scientific instrument are described below. Duplication is used extensively to ensure long term reliability.

Communications: the communications system consists of VHF transponders, S-band transmitters, r.f. power amplifiers and antennas. The characteristics of the VHF and S-band systems are summarised in Table 4. The S-band system is used only for transmission of telemetry data. The two transmitters can be connected to any of the four power amplifier-antenna combinations, but only one transmitter and one power amplifier may be selected at any one time and this will depend on which antenna has the most favourable view of the Earth. The VHF system consists of duplicate transponders and a four-element turnstile antenna and is used for the reception of ground-generated commands, the turn-around transmission of range and range-rate signals for tracking the spacecraft, and also to provide a backup telemetry down-link.

Table 4 Communications systems

	VHF	S-band
Transmitter frequency	136.860 MHz	2249.80 MHz
Power output	6 W	6 W
Modulation	PCM/FSK/AM	PM
Telemetry rate	800 b s ⁻¹	1.25 kb s ⁻¹ to 40 kb s ⁻¹ with fixed and reprogrammable formats
Antenna polarisation	Turnstile	Circular
Antenna pattern	Omnidirectional	60° conical
Receiver frequency	148.980 MHz	—
Receiver sensitivity	−106 db m	—

Commands initiated by the on-board computer or received directly from the ground are all processed by the two command decoders. Each can process 128 discrete commands and 48, 37-bit serial word commands.

Data handling: the data handling system is composed of the data multiplexer and the on-board computer. The data multiplexer serves as the spacecraft telemetry encoder and as the input data interface between the on-board computer and the rest of the spacecraft. 8-bit words are transferred to a serial data stream which is alternately made available to the ground and to the on-board computer using time sharing techniques. The telemetry bit rate is selectable by ground command from 1.25 to 40 kb s⁻¹. The on-board computer performs all attitude control computations and issues all reaction wheel torquing commands. It performs self-checks, monitors spacecraft performance safety functions, controls camera exposure times, and stores commands.

Stabilisation and control: the stabilisation and control system is used in orbital manoeuvres which include station keeping, pointing, and manoeuvring of the spacecraft. It has three different reference systems: (1) six gas-bearing, pulse-rebalanced, inertial-grade gyroscopes providing 0.01 arc s integrated rate resolution over 600 arc s per s; (2) star trackers (the FESs) in the scientific instrument which use the telescope optics to provide an angular resolution of 0.27 arc s throughout a 16 arc min field-of-view; (3) a two-axis digital Sun sensor which provides angular resolution to 15 arc s over a field-of-view of 64° × 124°. When a bright star is within 9 arc min of the target source, the guidance system uses the FES for position information and gyro system for rate damping. When a guide star is not available, precision-hold depends solely on a well-trimmed gyro reference with low frequency updates from a faint source.

Sequential three-axis manoeuvres from one star to the next use an integrated gyro reference resulting in accumulated errors of <4 arc min. The control system utilises a set of momentum-exchange reaction wheels for attitude control and relies on the hydrazine thrusters for momentum dumping.

Power: power is provided by two solar arrays and a highly efficient distribution and regulating system. During eclipse, and other periods when demand exceeds solar array output, power is provided through a boost regulator from two 6 amp h nickel-cadmium batteries.

Thermal control: the active thermal control system utilises louvres, insulation, heat pipes and heaters. Multi-nodal analysis of a thermal model and solar simulation tests were used to prove the design. The spacecraft may be divided into four sections, each with unique thermal requirements.

(1) The hydrazine bay which contains the hydrazine propulsion system and apogee boost motor and uses a combination of thermal blankets and active heaters.

(2) The main spacecraft compartment which is maintained between 0 °C and +40 °C with the batteries below 30 °C and with the gyros in the inertial reference assembly internally controlled at 57° ± 1 °C. Heat pipes attached to the main equipment platform, together with the louvres, keep gradients to <5 °C.

(3) The telescope, where heaters maintain the primary mirror near 0 °C and the focus mechanism between 0 and +30 °C. The primary mirror is conductively isolated from the telescope structure to reduce axial and circumferential gradients.

(4) The spectrograph, where the cameras are maintained between 0 and +20 °C. Temperature changes and gradients are minimised to prevent thermally induced misalignment. Satisfactory temperature control is obtained by enclosing the spectrograph within an aluminium shield to limit its heat exchange with the main spacecraft compartment and by temperature control of the primary mirror through which most of spectrograph's heat is dissipated.

Ground observatory control systems

Unlike previous unmanned astronomy spacecraft, IUE is operated in real-time by guest observers who generally lack detailed knowledge of the complex spacecraft and ground systems. Ground operating procedures have, therefore, been designed to allow the observer's research programme to be accomplished by selection from a library of modular pre-programmed operating sequences.

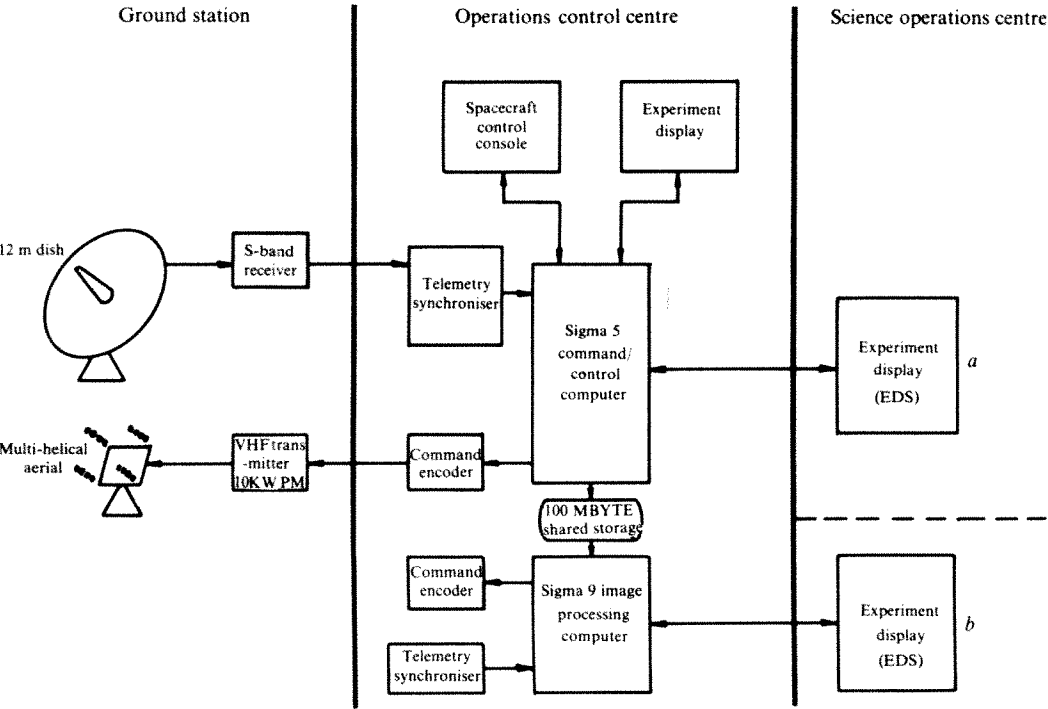


Fig. 7 US ground observatory. a, Observing centre; b, image processing centre.

IUE ground control is based on a large real-time computer software system to process telemetry and commands. Relatively complex spacecraft operations are accomplished by calling a series of operating procedures, each designed to accomplish a particular function, such as reading an image from a camera. Procedure execution is controlled by trained telescope operators. A computerised image processing system is then run offline to correct the astronomical images and produce a spectrum in absolute units as a function of wavelength¹³. The processing sequence consists of geometric and photometric correction, wavelength identification, data extraction and system efficiency correction and uses calibration tables derived from periodic analysis of calibration images.

The US ground observatory at GSFC is divided between three separate locations: the ground station, operations control centre, and science operations centre, see Fig. 7. The ground station maintains continuous radio communication with the spacecraft, relays telemetry data to the operations control centre and transmits command messages from the control centre to the spacecraft. The operations control centre contains a Xerox Data Systems (XDS) Sigma 5 computer used for real-time spacecraft operations and image acquisition, an XDS Sigma 9 computer used for image processing and data reduction, and a common disk storage unit. The Sigma 5 computer is operated from a spacecraft control console, consisting of a number of interactive

displays with keyboards used by engineers who continuously monitor the spacecraft performance. The science operations centre houses two experiment display systems (EDS) which are specially designed pseudo-colour image display and analysis consoles, containing their own mini-computers and disk storage. One EDS is used by the observer and telescope operator to conduct real-time observations and the other is used in image processing.

The ESA ground station and control centre is functionally identical with that at GSFC but incorporates a single Sigma 9 computer, with EDS, telemetry, command, and control consoles for operating the spacecraft. From this control centre the ESA- and SRC-sponsored observers use the IUE spacecraft for 8 h each day to conduct their research programmes.

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In-flight performance of the IUE

A. Boggess, R. C. Bohlin, D. C. Evans, H. R. Freeman, T. R. Gull, S. R. Heap, D. A. Klinglesmith, G. R. Longanecker, W. Sparks & D. K. West

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After the IUE's successful launch into an eccentric geosynchronous orbit, and following initial spacecraft checkout, observations were made of a set of high priority targets as an insurance against premature failure of the system. These were followed by the systematic performance evaluation of the various spacecraft and scientific instrument sub-systems, some of which were optimised in orbit. The optical performance of the telescope and spectrograph and the photometric performance of the SEC cameras used as spectrum detectors all seem satisfactory, but the ground data processing system although operational needs further development.

THE IUE was launched from the John F. Kennedy Space Center on 26 January 1978 into an eccentric geosynchronous orbit. The system is described in the previous article. The mission objectives required that the spacecraft be placed in a 24-hour orbit visible continuously from the NASA ground station at Goddard Space Flight Center and for at least 10 h per day from the ESA Villafranca Satellite Tracking Station near Madrid. This coverage allows the Goddard Control Center to control operations for 16 h each day and to monitor health and safety of the spacecraft while the Villafranca Tracking Station operates the satellite for 8 h each day. The orbital elements are summarised in Table 1. The satellite tends to drift slowly in longitude because of slight inaccuracies in the orbital period. However, the ascending node is kept near the longitude indicated in Table 1 by

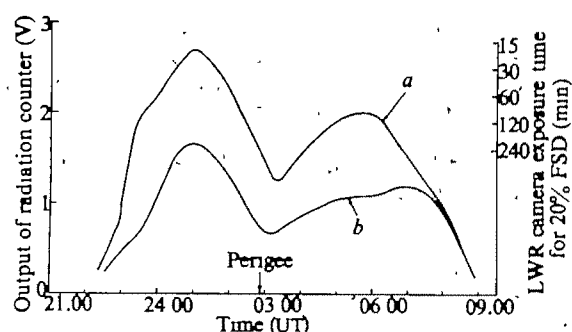


Fig. 1 Typical variation of electron flux (≥ 1 MeV) measured by the on-board counter. The counter output voltage, V , is related to the electron flux, F (electrons $\text{cm}^{-2} \text{s}^{-1}$), by $V = \log_{10} F - k$ where $k = 3.4$. The right hand scale gives the exposure time in minutes that would give 20% of full scale on the long wavelength redundant camera. This calibration was derived at several counter levels between 1.5 and 2.5 V. The short wavelength prime camera is 1.4 times more sensitive to radiation. a, 31 January–1 February, b, 30–31 January.

occasionally firing hydrazine thrusters. These small orbital adjustments will be required two to three times per year.

After the spacecraft was injected into the synchronous orbit, three-axis attitude control was established and the integrity of the basic spacecraft and scientific instrument systems was verified. At this point a selected set of high priority targets was observed so that some scientific data spanning various research areas would exist in the event of an early failure. An IUE science commissioning team, including project personnel and a few guest observers, had been formed to direct the early scientific checkout, observing, and performance evaluation activities. Each team member was supported by one or more associates so that the team had a large staff of scientists and engineers to assist in the commissioning tasks. The tasks covered three basic phases. (1) Immediately after turn-on, the selected high priority targets were observed to develop observing techniques and to collect some data in each of the broad research categories applicable to IUE. The observations are described in the following papers²⁻⁷ and fall into the categories of hot stars², cool stars³, interstellar medium⁴, X-ray objects⁵, extra-galactic objects⁶, and solar system objects⁷. (2) The scientific instrument and supporting spacecraft equipment were optimised. During this phase the telescope was focused, prime and back-up cameras were selected for each spectrograph, the settings of each prime camera were adjusted to achieve best performance, and the slewing and guiding capabilities of the spacecraft were improved. (3) The photometric properties of the scientific instrument were calibrated against standard stars, and other performance characteristics, such as image quality and spectral resolution, were studied.

At the end of these three phases routine guest observing began on 3 April 1978. The optimisation, evaluation, and calibration tasks were far too large to be fully completed within the available time. However, those items necessary for starting the routine observing phase were finished, and the basic procedures established whereby the scientific commissioning will be completed and maintained by the observatory personnel.

Spacecraft sub-systems

The spacecraft sub-systems whose proper operation are essential to the mission are described in the previous article¹. They include power, thermal, communications, data handling, and attitude control, and all are operating successfully. The power sub-system has performed according to its design since the launch. The solar arrays totally support normal operations over the acceptable range of β -angles (the angle between the telescope axis and the direction to the anti-Sun) from 0 to 135°. As the arrays degrade due to radiation damage the β -angle range for positive power will decrease, and the power system will begin to depend on stored energy from the batteries to maintain operation over all β angles. The batteries and power system

performed normally during the first eclipse period, during which the spacecraft passed through the Earth's shadow every day for about three weeks. Eclipse seasons for IUE, as for the Moon, occur every six months.

The overall thermal performance of the IUE spacecraft has been satisfactory. The only departures from pre-flight predictions are that the power sub-system electronics are running warmer than expected, and there is a larger than anticipated gradient between the two battery packs. There was some initial concern about how the batteries would perform under load with this gradient; however, no problems arose during the first eclipse period. The temperatures throughout the scientific instrument are close to the predictions and the scientific instrument heaters are routinely cycled to keep its thermal conditions within the desired range.

The spacecraft communication and data-handling sub-system has been used in all its various formats and bit rate configurations, and it operates flawlessly. Most operations use a telemetry rate of 20 kbits s^{-1} . The command sub-system is performing properly with hundreds of commands issued to the spacecraft each day to support routine real-time operations. Occasional command-verify anomalies have been observed, but in most cases these have been traced to ground transmitter adjustment errors. Occasional interference is encountered from other NASA satellites during ranging measurements. The primary on-board computer has been in operation since spacecraft turn-on. During the first few weeks several instruction failures were observed which occasionally caused the computer to halt. However, software modifications were installed to detect such conditions and recover automatically without impact on scientific operations. While the primary function of the computer is attitude control, its other functions include timing camera exposures, and monitoring certain spacecraft parameters. As with the other spacecraft systems, a redundant on-board computer is available in case the prime system should fail.

The attitude control sub-system operates satisfactorily in all modes of operation. It controlled nutation and precession while the spacecraft was spinning in the transfer orbit and, after injection into the synchronous orbit, it executed the de-spin and Sun acquisition manoeuvres. Three-axis control was established using the on-board computer, the inertial reference assembly, and the reaction wheels. The initial attitude reference was obtained by measuring the solar position with fine Sun sensors in pitch and roll and the approximate Earth position with a panoramic attitude sensor in yaw. The spacecraft was then slewed to point the telescope toward the North Ecliptic Pole, where a star field was identified using the telescope and star tracker. Manoeuvres to new positions are performed, one axis at a time, by inserting in the on-board computer new gyro references relative to the known spacecraft orientation. Reaction wheels slew the spacecraft, and hydrazine reaction jets are fired once or twice daily to reduce wheel momentum that accumulates due to disturbance torques on the spacecraft. Typical pointing errors after large angle slews have been 1–2 arc min, which is well within the 16 arc-min field of the telescope. The star tracker in the scientific instrument can identify and guide on stars brighter than 14 mag. Using the star tracker for guidance, pointing accuracies ~ 0.2 arc s have been achieved. On the rare occasions when there is no suitable guide star in the field, the gyro drift rates may be nulled while the tracker is guiding on a nearby star and then the gyros may be

Table 1 Initial orbital elements

Semi-major axis	42,152 km
Perigee	32,050 km
Apogee	52,254 km
Eccentricity	0.240
Inclination	28.6°
Right ascension, ascending node	208.1°
Argument of perigee	257.1°
Period	23h55min33s

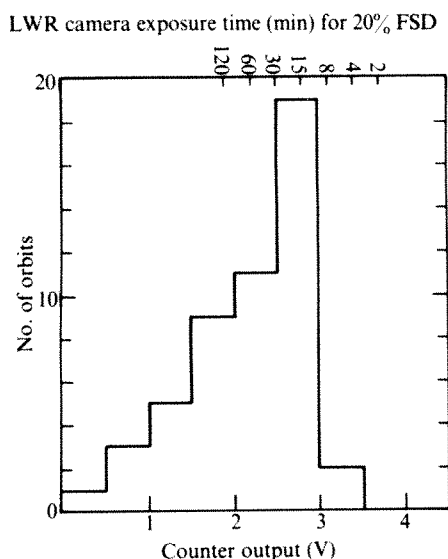


Fig. 2 Histogram showing the variation in peak flux during each of 50 orbits for which data are available for the period 29 January–10 April 1978.

used for inertial guidance with random drifts ~ 3 arc s per h. If moving targets are to be observed, the gyro rates can be adjusted to track the target's motion.

Optical performance

Measurements of telescope image quality before launch indicated that the ratio of transmission between the small spectrograph entrance aperture (3 arc s) and the large spectrograph entrance aperture (10×20 arc s) was 0.65 at 20°C , 0.60 at 5°C , and 0.55 at -10°C . Because of air turbulence and the angular diameter of the laboratory source, these measurements were considered to be lower limits of achievable performance.

Thermal conditions have been adjusted in orbit and are being controlled actively to maintain a nominal primary mirror temperature of -3°C . This should allow pointing to any object outside the 43° Sun avoidance cone without significant changes to telescope focus. After refocusing the telescope in orbit, spectrograms of the same star obtained using the large and small apertures in the short wavelength spectrograph have indicated small aperture transmissions of up to 70%. Spectra of stellar images in the large aperture are ~ 5 arc s full width at half maximum, perpendicular to dispersion.

Sources of scattered light in the telescope may be divided into three categories: the Sun; Earth and Moon; and bright stars. The IUE telescope baffles (see ref. 1) were designed to eliminate sunlight over the region of sky outside the 43° half-angle Sun avoidance cone. No evidence of scattered sunlight has been observed at angles of 50° or more from the Sun. At angles between 45 and 50° , stray sunlight is barely detectable in the star trackers. There are no restrictions on observing near the Earth or Moon, but scattered light may affect the operation of the star trackers. Observations of stars with no evidence of scattered light contamination have been made within 25° of the Moon and within 25° of the Earth's limb. The practical limit has not yet been fully determined, but when the Earth is within the 16 arc min field-of-view, the star tracker output is saturated in every operational mode. Observations of Jupiter and its satellites have shown that 6th mag objects can be observed and tracked within 90 arc s of a -1.7 mag object. This performance would scale to tracking a 14th mag object at 90 arc s from a 6.3 mag object. It is also important to know how severely the spectrum of an object can be contaminated by scattered light from a bright star located close to it. Tests on η UMa show attenuations of 10 stellar magnitudes when the star is 10 arc s from the small entrance aperture of the long wavelength spectrograph and 12 stellar magnitudes at 20 arc s.

The resolutions of the long wavelength and short wavelength spectrographs have been determined from spectra of the Pt–Ne

hollow cathode lamp that is used on-board for wavelength calibration. At high dispersion in the short wavelength spectrograph, the full width at half maximum (FWHM) of a spectral line from the Pt–Ne lamp is $0.085 \text{ \AA}$ at $1,150 \text{ \AA}$, and it increases linearly with wavelength to $0.19 \text{ \AA}$ at $2,100 \text{ \AA}$. In the long wavelength spectrograph, $\text{FWHM} = 0.20 \pm 0.01 \text{ \AA}$, constant with wavelength. The data for the short wavelength spectrograph correspond to a resolving power, $R = \lambda/\text{FWHM} \approx 1.2 \times 10^4$, as was expected. The data for the long wavelength spectrograph give resolving powers that vary from 1.0×10^4 to 1.5×10^4 over the wavelength range rather than the approximately constant value of 1.3×10^4 that was expected. The reason for this is not yet understood. The spectral resolution measured from Pt–Ne spectra in the low dispersion modes is $\leq 7 \text{ \AA}$ in the short wavelength spectrograph and $\leq 8 \text{ \AA}$ in the long wavelength spectrograph. These results are upper limits because all features of the Pt–Ne spectrum observed at low dispersion are, to some extent, blends of several lines.

The on-board Pt–Ne lamp⁸ serves two purposes. First, it defines the spectral format to guide the extraction of data from the spectrum and its local background; this is clearly a more intricate process in the high dispersion mode with its 2-dimensional format. Second, it assigns a wavelength to each data point of an extracted spectrum. The procedure involves two steps. First, the emission line spectrum of the lamp is obtained, and the positions of the spectral lines on that image are found using a cross-correlation technique. In the case of the high dispersion spectra ~ 200 spectral lines are located, while in the case of low dispersion spectra the number is ~ 15 – 20 . Second, a multivariable regression analysis is performed to relate the sample s and line l positions to their wavelength λ and order numbers m . The equations of condition are given below for high dispersion:

$$s = a_0 + a_1 m \lambda + a_2 (m \lambda)^2 + a_3 m + a_4 \lambda + a_5 m \lambda^2 + a_6 m^2 \lambda$$

$$l = b_0 + b_1 m \lambda + b_2 (m \lambda)^2 + b_3 m + b_4 \lambda + b_5 m \lambda^2 + b_6 m^2 \lambda$$

and for low dispersion

$$s = a_0 + a_1 \lambda \quad l = b_0 + b_1 \lambda$$

These dispersion constants are then used to define the spectral format of the target spectrum. The errors in this procedure are assessed in Table 2, which shows that the largest uncertainty in assigned wavelengths arises in transferring the dispersion constants derived from a Pt–Ne spectrum to the image of a stellar spectrum. These errors can be removed by identifying a feature in the astronomical source and applying an appropriate correction.

Table 2 Sources of error in wavelengths

Source	High dispersion	Low dispersion
Error in laboratory wavelength	$< 0.004 \text{ \AA}$	$< 1 \text{ \AA}$
Error in measured positions of spectral lines	$< 0.07 \text{ \AA}$	$2 \text{ \AA}$
Inadequacy of dispersion formulae to represent spectral format	$< 0.07 \text{ \AA}$	$< 2 \text{ \AA}$
Error in transferring dispersion relations to target spectra	$0.2 \text{ \AA}$	$6 \text{ \AA}$

Camera performance

Before launch an extensive programme was undertaken to investigate the effect on camera performance of the settings of the camera-tube and deflection-coil operating parameters⁹. This programme resulted in a set of values for the commandable voltages that was used immediately after launch to observe the high priority targets. After the high priority observing period, time was allocated to check the commandable camera settings, using the performance before launch as a baseline. In addition, the post-launch check on the operating parameters was extended to include the implementation of a new target preparation sequence, called SPREP, which had been developed during additional laboratory testing of the flight spare cameras.

Table 3 Camera performance

Camera	Photometrically corrected 40% image				Random noise on pedestal			Preparation sequence
	Mean S/N* (Single pixel)	Min. S/N (Single pixel)	Mean S/N (3 × 3 pixels)	Photometric† stability	Mean σ‡ (Single pixel)	Peak σ (Single pixel)	Mean σ (3 × 3 pixels)	
Long wavelength redundant	10.0	5.8	21.2	±7%	3.0 DN	7.9 DN	1.1 DN	TPREP: ground calibration
	13.4	8.0	29.0	±2.5	1.4	2.7	0.4	SPREP: after re-optimisation
	8.7	5.5	19.4	±5.5	2.9	6.0	1.0	SPREP: in-orbit ITF
	7.8	3.5	19.2	±7.5	2.7	5.7	0.9	FPREP: after re-optimisation
Short wavelength prime	12.2	4.0	30.2	±13.5	1.6	2.8	0.7	TPREP: ground calibration
	10.5	6.5	23.1	±2.5	1.4	2.9	0.5	SPREP: after re-optimisation
	10.9	6.6	25.0	±3.2	1.7	3.6	0.6	SPREP: in-orbit ITF
	11.1	6.0	25.0	±5	1.3	2.2	0.6	FPREP: after re-optimisation

All results refer to the low gain setting of the head amplifier with the SEC tube at maximum gain.
* The mean signal-to-noise (S/N) ratio is averaged over the whole target area for both individual pixels and for 3 × 3 pixel blocks. The minimum S/N ratio is the mean over a block of 24 × 24 pixels in the area of worst noise.
† The photometric stability represents the peak-to-peak spread in signal level after photometric correction has been performed.
‡ The mean standard deviation (σ) is averaged over the whole target area for both individual pixels and for 3 × 3 pixel blocks. The peak standard deviation is the mean over a block of 24 × 24 pixels in the area of worst noise performance.

SPREP has now replaced the original preparation sequence, TPREP, which was used during high priority observing. Further images were obtained to serve as a new baseline for measuring long term drifts in camera performance. Two other target preparation sequences, also initially developed in laboratory testing, have been implemented. These are FPREP, a fast preparation sequence designed for use when speed of operation is important and some degradation in image quality can be tolerated, and XPREP, which can be used to remove residual effects of over-exposed images.

The function of the preparation sequences is to remove all trace of previous images and to leave the target with a uniform low-level low-noise pedestal. Target preparation involves a saturation exposure of the camera, using tungsten floodlamps, and read out of the charge, followed in the case of TPREP and SPREP by a lower level flood exposure and subsequent read. The original preparation sequence, TPREP, has the disadvantage that beam pulling effects can lead to small scale instabilities in the action of the read beam, particularly when reading out the saturated exposure of the preparation sequence. These beam instabilities result in patches of increased noise and poor photometric stability. The new sequence, SPREP, gives significant improvements in photometric stability and considerably reduced noise on low-level signals. The improvements are

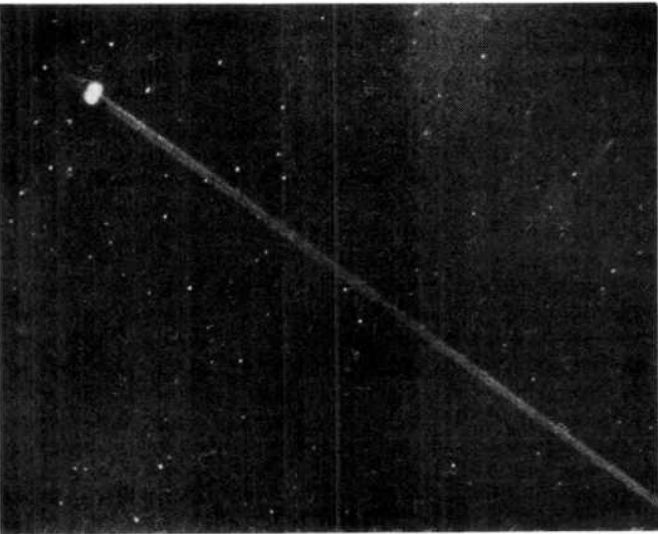
achieved principally by scanning the target with a defocused beam and with carefully chosen alignment settings, permitting a much more stable scanning action. Other improvements in noise performance are obtained by making the second floodlamp exposure at a lower gain setting, thus reducing the effect of photon noise, and by increasing the level of this exposure from 20% of saturation to 50%, at which level the random noise on the pedestal has been shown empirically to pass through a minimum value.

Table 3 lists the camera performance before and after reoptimisation. The performance using SPREP is significantly better than that for TPREP. The photometric stability is considerably improved, the signal-to-noise ratio in the worst areas has been improved, and the random noise on the pedestal has been reduced, particularly in the case of the long wavelength redundant camera. Also shown in Table 3 is the camera performance derived from the photometric calibration images taken shortly after the end of the reoptimisation programme. The performance of the short wavelength prime camera is comparable with that determined from the evaluation images taken as part of the reoptimisation work. However, in the case of the long wavelength redundant camera the performance seems to have degraded. The reason is not yet fully understood and more analysis remains to be completed. The apparent change may be a result of temperature transitions in the region of the detector or associated electronics which could cause a slight shift in the position of individual pixels on the scanning raster and hinder the removal of fixed pattern noise.

FPREP, the fast target preparation sequence, is achieved using a single floodlamp exposure and carrying out the read operations at a scanning rate of 320,000 pixels s⁻¹, which is 64 times the speed of the fastest scanning rate used in other modes and is considerably faster than the response time of the line deflection digital-to-analogue converter. The beam is not pulsed as it is in normal operation, but is held on continuously during the scan and the staircase waveform applied to the deflection coil is smoothed into a slope on which are superimposed spikes resulting from 'glitches' in the digital-to-analogue converter. These spikes give rise to a series of vertical bars, typically about 10% of saturation, superimposed on the image and degrading the quality of the data. It had been thought that these bars would not be stable with time, preventing their removal by means of photometric correction, and Table 4 shows that FPREP is significantly inferior to SPREP in the case of the long wavelength redundant camera, though still quite adequate for its intended use. The performance of FPREP for the short wavelength prime camera compares favourably with that using SPREP. It remains to be seen whether the bars are stable in the long term.

XPREP, which is used to remove the residual components of highly over-exposed images, is similar to FPREP, except that the floodlamp exposure level is four times larger, driving the

Fig. 3 Contrast-enhanced but otherwise unprocessed low-dispersion short wavelength spectrum of the faint ($B \sim 14$ mag) extragalactic object B2 1101+38 exposed for 4 h. The geocoronal Ly α emission fills the 10×20 arc s aperture and can be distinguished from the narrower object spectrum. There is no sign of background from other atmospheric emissions either in this or the long wavelength spectrum. The numerous bright spots are generated in the detector. The comet-like image near top right is a cosmic-ray induced event occurring in the detector optical components.



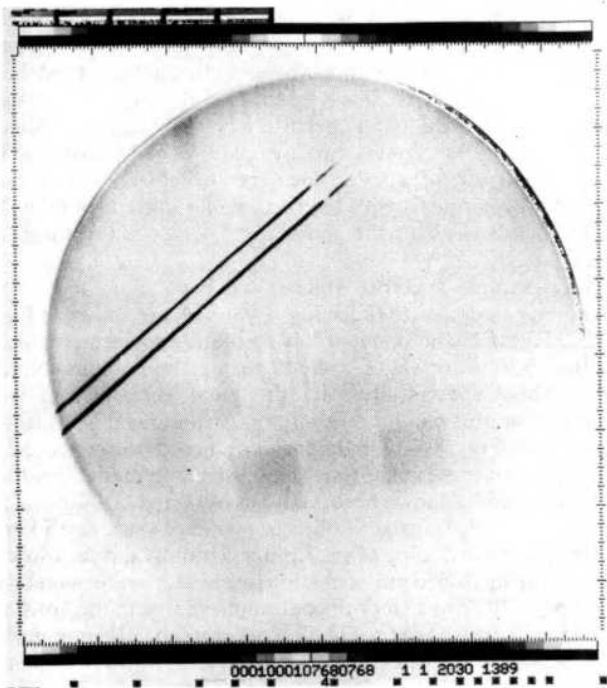


Fig. 4 Photographic representations of a raw camera image showing two low resolution short wavelength spectra of θ^2 A Orionis (O9.5 Vp, $V = 5.07$). The upper spectrum was exposed for 1 s through the large aperture and the lower spectrum was exposed for 4 s through the small aperture. The spectra extend from $\sim 2,000$ to $1,150$ Å. The strong interstellar Ly α absorption line at $1,216$ Å is clearly seen at the short wavelength end of each spectrum.

target well into saturation and completely obliterating any residual images. XPREP is normally followed by SPREP in order to obtain a reproducible low-noise pedestal. It has been used on both the long and short wavelength cameras following exposures containing spectral lines that were over-exposed by factors of up to 1,000. Initial results show that all traces of residual image are removed, while control exposures using SPREP only show residual signal levels of several per cent in line images that were over-exposed by factors of $\sim 1,000$.

The efficiency of IUE depends on the amount of overhead time associated with each exposure. In addition to the times for slewing and for target acquisition there are overheads associated with the operation of the cameras. As shown in Table 4 these fall into the categories of camera hardware overheads and ground control computer overheads. Considerable improvements were made to the latter during commissioning and further improvements are expected in the procedures by which the computer controls camera operations. Table 4 shows overheads for the two most commonly used telemetry rates, 20 and 40 kbits s^{-1} . It is often possible to hide the overhead associated with SPREP and READ by performing them during a long exposure in the other spectrograph; SPREP may also be carried out during a slew.

Background corrections

There are several contributors to the background which are apparent in camera images. The first of these is the null pedestal discussed in the previous section. This pedestal is a zero offset, typically amounting to 15% of saturation, that must be sub-

Table 4 Overhead time associated with camera operations					
Operation	Execution of procedure in ground computer	Camera hardware time		Total overhead	
		at 20 kbit s^{-1}	at 40 kbit s^{-1}	at 20 kbit s^{-1}	at 40 kbit s^{-1}
SPREP	10 m	13 m	7 m	23 m	17 m
FPREP or XPREP	4 m	1 m	1 m	5 m	5 m
EXPOSE	4 m	0 m	0 m	4 m	4 m
READ	10 m	5.5 m	3 m	15 m	13 m

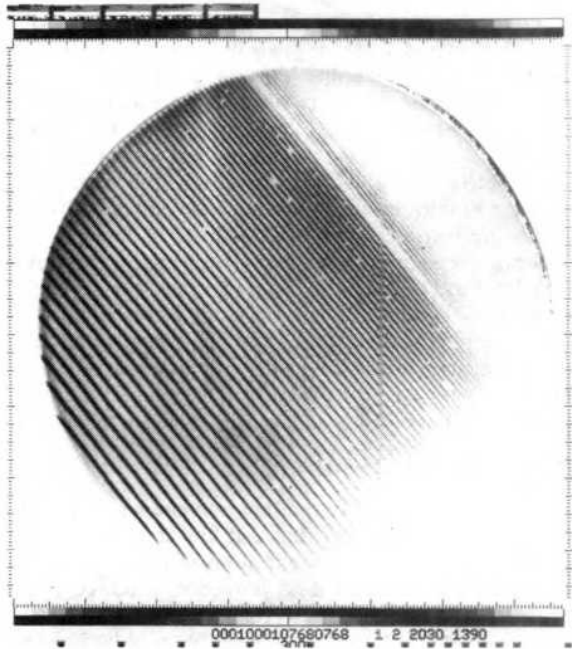
Table 5 Sample 1- σ noise in extracted spectra				
Camera	Object	Image	σ_{meas} (%)	σ_Q (%)
1	ζ Oph	LWP1031	4.2	1.9
2	η UMa	LWR1172	2.8	2.3
3	η UMa	SWP1164	<2.4	2.1
4	ζ Oph	SWR1031	3.6	1.8

tracted from each image. Although there is in principle a time-dependent internal tube noise due to thermal effects and to phenomena such as phosphorescence in the image converter, in practice the tube background appears to be negligible for exposures as long as 5.5 h, which is the longest integration time attempted so far.

During the passage of the satellite through the Earth's outer belt of trapped electrons there is often a significant background level in the data due to Cerenkov radiation in the input window. There is apparently no effect on the stored image in the SEC tube when the camera is in the STANDBY or READ modes. A silicon diode ionisation counter on board the spacecraft serves as a real time monitor of the flux of electrons ≥ 1 Mev, which are those expected to penetrate the radiation shielding and produce Cerenkov radiation. Figure 1 shows the variation of the output of the counter during parts of two typical orbits. The main maximum always occurs before perigee as the satellite passes just above the peak of the outer belt on its way south. The second maximum occurs as the satellite passes north at a greater altitude. The result of the in-orbit calibration of the response of the cameras to this radiation is also shown in Fig. 1. Using this calibration and the real time output from the radiation counter the observer can arrange to terminate his exposure at a suitable background level.

Figure 2 shows the statistics of the day-to-day variation in maximum radiation flux. From this figure it will be seen that for $\sim 40\%$ of the time exposures of over 2 h are possible during the passage through the belt. During the remaining 60% of the orbits, there will be restrictions on the maximum exposure time for periods between 2 and 8 hours duration. The radiation level in the belt is expected to increase as the peak of solar activity is approached.

Fig. 5 Photographic representation of a raw camera image showing a high resolution short wavelength spectrum of θ^2 A Orionis. The exposure was for 5 min through the small aperture. Echelle orders in the lower left portion of the figure provide wavelength coverage to $\sim 2,100$ Å while orders in the upper right provide wavelengths down to $\sim 1,150$ Å. The broad interstellar Ly α absorption line at $1,216$ Å nearly eliminates two complete echelle orders. Numerous narrow interstellar lines can be seen but they should not be confused with the rectangular pattern of fiducial marks.



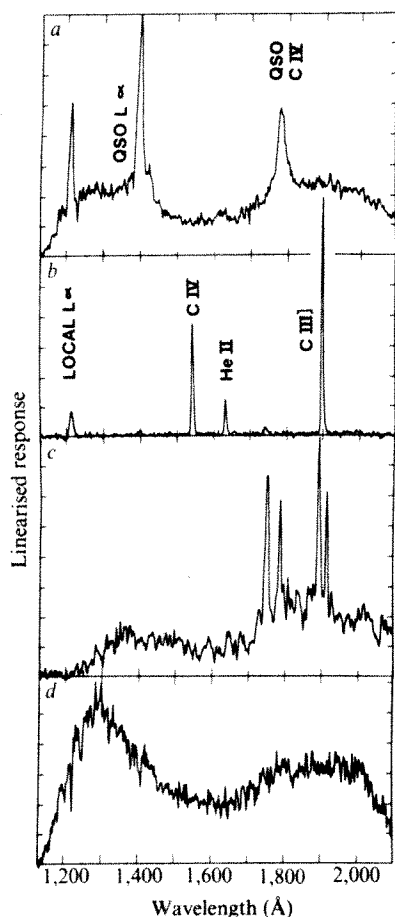


Fig. 6 A selection of low dispersion short wavelength spectra obtained by IUE during the commissioning period. Some of the strongest lines are identified. *a*, 3C273; *b*, NGC7027; *c*, η Car; *d*, BD+75°325.

The only detectable sky background source is geocoronal Ly α emission. This is evident in the example shown in Figure 3, which is a contrast-enhanced, low dispersion spectrum taken with the short wavelength redundant camera of the faint ($B \sim 14$ mag) extragalactic object B2 1101+38 exposed for 4 hours during the commissioning phase. Here the geocoronal Ly α emission, which fills the 10×20 arc s entrance aperture, can be distinguished easily from the object spectrum, which appears as a point source. There is no sign of background from atmospheric continuum emission or from other line emissions, such as the O I multiplet near λ 1304 or [O I] λ 1356, which are known to peak far below the satellite altitude. There are some bright spots generated in the detectors themselves; residual optical 'hot spots' may fluoresce in the phosphor of the UV converter following the flooding of the preparation procedure, and phosphor radioactive decay spots and internal ion events appear occasionally. Finally, cosmic-ray induced events, which exhibit a comet-like appearance, may occur in the detector optical components. These are distinct from the diffuse Cerenkov radiation background caused by trapped radiation. Cosmic rays usually produce small clusters of saturated pixels that must be ignored in analysis of the data. Clearly, it is important to use the basic pictorial data to distinguish real from spurious lines by verifying that any apparent feature is the same width as the actual spectral image.

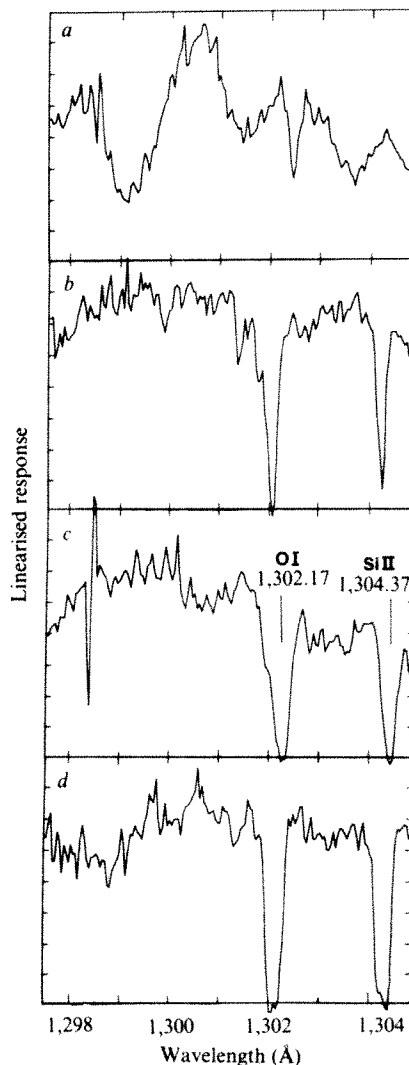
Photometric performance

Figures 4 and 5 show representative raw images obtained with the short wavelength prime camera at low and high dispersion. These are photographic representations of the original camera images and are a standard data reduction product. For both figures the object is θ^2 A Orionis (O9.5 Vp, $V = 5.07$), a bright early type star in the vicinity of the Orion nebula. For the low

resolution exposure in Fig. 4, spectra were obtained through both entrance apertures and the resultant spectra extend from $\sim 2,000$ to $1,150$ Å. At the short wavelength end the interstellar Ly α line at $1,216$ Å is clearly seen. For the high dispersion spectrum in Fig. 5 the small aperture was used. The interstellar Ly α line nearly eliminates two complete echelle orders and numerous narrow interstellar lines are also clearly visible but should not be confused with the rectangular pattern of fiducial marks which is used for the geometrical correction of the raw images.

Several sample spectra, which have been extracted from camera images similar to those just discussed, are shown in Figs 6 and 7. Figure 6 contains four low resolution spectra obtained with the short wavelength camera during the commissioning period. These spectra illustrate the great versatility of the instrument in observing a diversity of astronomical objects. A dynamic range of 18 mag in the incident flux can be covered by using appropriate exposure times and dispersion mode. In addition to the samples shown here, IUE has obtained UV spectra of cool stars, other extragalactic objects, standard stars, and Solar-System objects, including Mars, Jupiter, Uranus, and the Moon. Spectra ranging from a pure emission line object in the planetary nebula NGC7027 to a smooth continuum source in the hot star BD+75°325 are shown in Fig. 6. The presence of some noise due to quantum statistics and residual system noise is evident,

Fig. 7 A selection of high dispersion stellar spectra from order 106 of the short wavelength echelle spectrograph. The two strong absorption lines of O I and Si II are primarily interstellar. The glitch on the left in HD153919 is produced by one of the camera fiducial marks which are about 3 pixels wide. *a*, η UMa; *b*, ζ Oph; *c*, HD153919; *d*, HD93521.



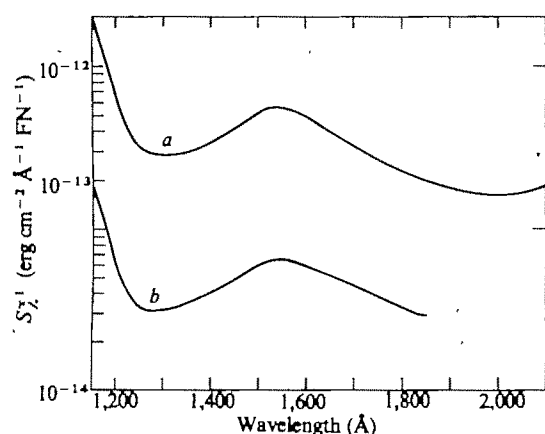


Fig. 8 Inverse absolute sensitivity curves for the short wavelength cameras in low dispersion. Multiplication of the indicated values by the linearised flux numbers obtained from the COMPARE data extraction program and division by the exposure time yields absolute fluxes for continuum sources in $\text{erg cm}^{-2} \text{s}^{-1} \text{\AA}^{-1}$. The curves are for the short wavelength redundant camera as used during the high priority observations and for the re-optimised short wavelength prime camera as it is now being used for routine observing. As the data reduction system scales fluxes differently for the two cameras, these curves should not be used directly for estimating exposure times. The real sensitivity difference between the prime and redundant cameras is only about a factor of two. *a*, SWR(4); *b*, SWP(3) reoptimised

particularly in BD +75°325. The emission at 1,216 Å is typically present in long exposures at low dispersion and is due to resonant scattering of solar Ly α in the geocorona as discussed earlier. Note that in the quasar 3C273 the intrinsic Ly α and C IV 1,550 Å emissions are shifted longwards by the redshift $z = 0.158$.

Part of order number 106 from the short wavelength echelle spectra of four stars is shown in Fig. 7. Strong interstellar lines of O I 1,302.17 Å and Si II 1,304.37 Å are evident. Some variation in line position exists, because wavelengths are not corrected for velocities external to the spacecraft and because η UMa and HD153919 were observed in the large aperture, where pointing uncertainties cause wavelength errors. In η UMa, note the stellar features that are broad and shallow, in contrast to the sharp interstellar lines. The background used to find the net response in Fig. 7 was measured between the orders, where there is some residual due to lack of complete separation of the echelle orders at these wavelengths. Consequently, the cores of the interstellar lines tend to lie below zero. Improvements in the data processing system will be needed to correct this.

There are at least seven sources of noise in the IUE data: (1) quantum noise in the detected photoelectrons; (2) noise in the individual intensity transfer function (ITF) curves used to linearise the raw data values and reduce them to flux units; (3) inaccuracies in aligning the stellar images with the flat field images of the ITF sequences, so that each pixel uses the exact ITF curve intended; (4) random system noise in the tube readout and telemetry; (5) pseudo-periodic noise from other spacecraft subsystems; (6) noise in the pedestal level, which is the zero signal charge on the target after the camera preparation sequence, and (7) radiation background. The radiation background is discussed above and can be neglected for the short exposures described here. Care must be exercised to subtract in a smooth way any backgrounds present in the gross signal; otherwise, additional noise can be introduced by the data reduction. Table 5 lists some one- σ noise levels per sample point in IUE gross extracted spectra, where σ_{gross} includes noise from sources 1-6. The values of σ_0 are the quantum noise expected from the number of photoelectrons required to produce a net signal of 90% of saturation. For the spectrum of η UMa obtained with the long wavelength redundant camera, the measured noise approaches the theoretical value. In the other cameras, the measured noise is about twice the theoretical limit.

The low resolution IUE data best define the photometric reproducibility of the IUE system, because the background can

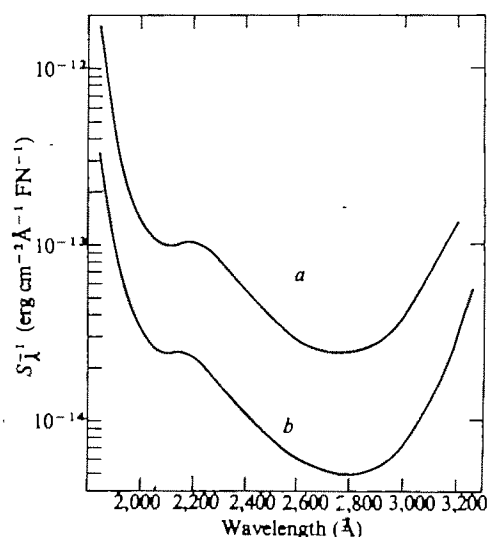
be measured reliably on either side of the spectrum. Studies of the photometric stability were therefore made by taking low dispersion spectra of the same star (HD50753, B3 IV, $V = 6.7$ mag) at periodic intervals in the large slot of each spectrograph. These data were obtained from 21 March-15 May 1978, and indicate a change in the sensitivity of the system. Over the full 55 days, the data showed decreases in sensitivity of 5% at 1,400 Å and 12% at 1,700 Å (taken with the short wavelength prime camera) and decreases of 5% at 2,150 Å and 9% at 2,750 Å (taken with the long wavelength redundant camera). The reasons for these changes are not yet understood and they will continue to be investigated as part of an overall programme to maintain a photometrically calibrated system.

Preliminary absolute sensitivity curves for low resolution IUE spectra have been obtained by observing hot stars with absolute energy distributions established from previous satellite and rocket observations. This procedure was preferred to a pre-launch calibration, as major efforts have been undertaken in the UV to calibrate existing satellite data¹⁰. The only extensive set of UV flux measurements available that covers the entire wavelength range spanned by IUE is the absolute OAO 2 spectrophotometry^{11,12}. Therefore, we have chosen to relate the preliminary IUE sensitivity curves to the absolute spectrophotometry on the OAO 2 system. Figures 8 and 9 show preliminary inverse sensitivity curves for all four cameras. The curves for the long wavelength redundant and short wavelength prime cameras apply to their re-optimised performance. The curves for the long wavelength prime and short wavelength redundant cameras are as they were used during the high priority phase of the scientific commissioning. The quantity plotted, S_{λ}^{-1} ($\text{erg cm}^{-2} \text{\AA}^{-1} \text{FN}^{-1}$), and the absolute flux, F_{λ} ($\text{erg cm}^{-2} \text{\AA}^{-1} \text{s}^{-1}$), are related by

$$F_{\lambda} = S_{\lambda}^{-1} \frac{(\text{FN})}{t}$$

where t is the exposure time in seconds and the IUE flux number (FN) is the linearised extracted net spectrum. Because of the details of the linearisation that produces FN, the sensitivity curves do not represent actual differences between cameras. The curves plotted are for large aperture exposures and a 10 pixel high spectral extraction with the program COMPARE. For small aperture exposures, where the light loss will depend on the telescope focus and the object positioning these curves can only be used in the relative sense. The curves are based on short exposures (~ 0.6 -2 s) of μ Columbae (HD38666, $V = 5.17$) timed with the aperture select mechanism in order to avoid uncertainties associated with the rise and fall times of the camera high voltage. The timing uncertainty associated with this

Fig. 9 As for Fig. 8 but for the long wavelength cameras *a*, LWP(1), *b*, LWR(2) reoptimised.



mechanism may approach 10% for the shortest exposures. The sensitivity curves of Figs 8 and 9 imply short and long wavelength limits at about 1,150 and 3,200 Å, respectively. These sensitivity curves were obtained using preliminary intensity transfer curves to linearise the raw camera responses and a data extraction system that requires revisions. They should permit the transformation of IUE data into the OAO 2 spectrophotometric system with a relative accuracy of ~10%. However, the final result is also subject to the additional uncertainties of the OAO 2 absolute calibration, which seems to be accurate to about $\pm 5\%$ for $\lambda > 1,800$ Å and $\pm 20\%$ for $\lambda < 1,800$ Å. Figure 10 shows the result of using the derived short wavelength redundant camera calibration to compute a stellar flux distribution. The bump near 1,500 Å is located just where the OAO 2 flux distributions lie furthest above the measurements of the TD-1 satellite and other experiments¹⁰.

Because of its importance, a completely independent calibration, based on the same principles as above but using the TD-1 (S2/68) absolute fluxes as standard, was carried out during the commissioning phase. This is described by Boksenberg *et al.*⁶, and shows differences from that above (for example, it does not produce a bump near 1,500 Å) and further work is needed to establish the best sensitivity curves for general IUE use. In the work described in the following papers, both of these early calibrations have been used, that based on TD-1 for the interstellar⁴ and extragalactic⁶ work, and that based on OAO 2 for the rest^{2,3,5,7}.

Data reduction

Figure 11 shows the flow of the various types of images through the major steps of the data reduction system. Wavelength calibration lamp images are the emission line spectra from the on-board Pt-Ne source. Sequences of flat field images allow the nonlinear intensity transfer function (ITF) to be measured for each pixel in the image field. The standard star images are used to determine the absolute instrumental sensitivity by comparison with previous rocket and satellite spectra.

The first manipulation performed on each image is a geometrical transformation to make each pixel in each image

Fig. 10 a, The IUE spectral response for HZ43 produced by the image processing system after ITF correction. b, The absolute flux estimate for the object after application of the preliminary absolute calibration.

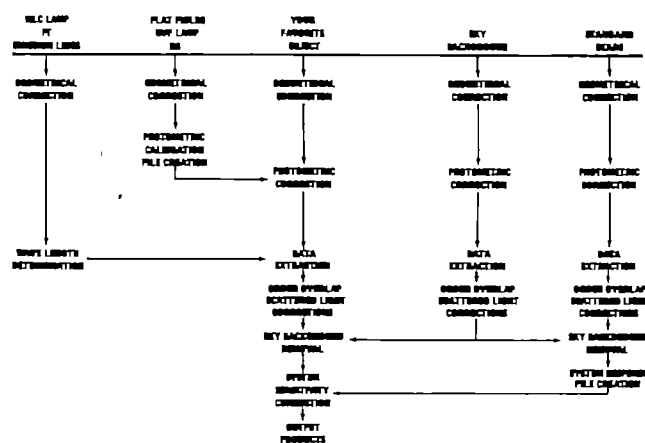
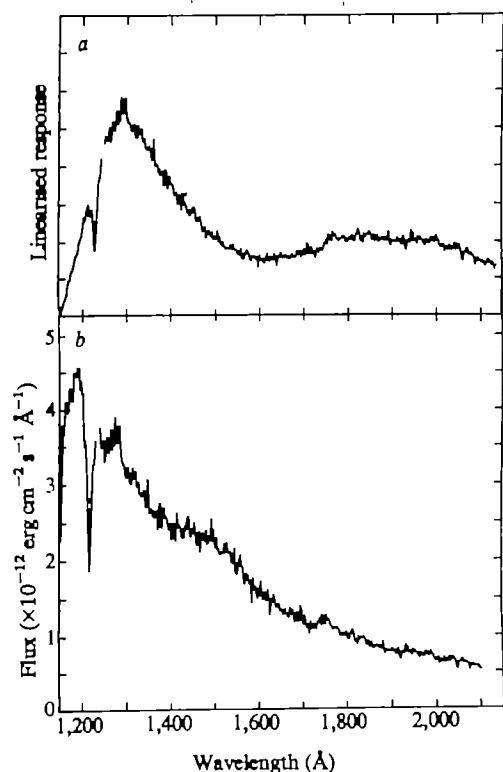


Fig. 11 The flow of images through the IUE spectral image processing system, showing the major image processing and data reduction tasks to be performed on each type of image.

refer to the same point in the focal plane at the camera faceplate. This removes the distortion inherent in the electron optics of the SEC tube, and hence the spectral orders are straightened out so that simpler fitting functions can be used to find the orders. However, in doing this transformation the data have been resampled, which results in some smoothing, each pixel in the transformed image being the linearly weighted average of the four adjacent pixels in the raw image. The next step is the photometric or ITF correction, which involves each point in the 768×768 pixel image being transformed with its own ITF curve. There are 12 levels of exposure in the ITF tables, with multiple images averaged together at each exposure level to reduce noise. Each pixel of the image being reduced is corrected by interpolation in its own 12-point table. The result is an image that is related linearly to the input flux on the camera, but has had no wavelength-dependent sensitivity corrections applied to it.

The next step is to extract the spectral data from the images. The location of the spectrum must be determined and this is obtained from known emission lines in the spectrum of the Pt-Ne calibration lamp, whose position, order number, and wavelength are fitted to the dispersion equations given above. When spectra are taken through the large entrance aperture, the wavelength scale derived from Pt-Ne calibration images obtained through the small aperture must be shifted linearly by about 30 pixels. In this case, the zero point of the resulting wavelengths may be slightly in error due to uncertainties in the exact centering of the target image within the 10×20 arc aperture. After wavelengths have been assigned, the extraction of the spectrum is accomplished by passing one pseudo slit with a height of 5 diagonal pixels along the orders and another pseudo slit 1 diagonal pixel high between the orders. The inter-order background is smoothed and subtracted from the spectrum. This net spectrum must still be corrected for the system sensitivity. In high dispersion, a 'ripple correction' is applied to remove the effect of the echelle blaze. The data are then multiplied by the system sensitivity curves derived from standard stars.

Several problems remain in the data reduction as of May 1978, one of the major ones being the proper separation of the high dispersion orders. In the extraction of the high dispersion spectrum the smoothed interorder background is subtracted. This is adequate for the orders that are far enough apart for the inter-order background to be a true measure of the local background. However, as the order separation decreases at the shorter wavelengths, the inter-order background becomes an over-correction in both spectrographs. A major complication is that a variation of step size caused by the dynamic refocus voltage in the cameras makes the echelle orders deviate from straight lines, even in a geometrically corrected image. Therefore, in the refocus regions the pseudo slits that track the spectrum and the interorder background tend to lose or gain

signal, respectively. Thus, a small dip of typically 10% in an extracted spectrum becomes an even larger error when the anomalously high inter-order background is subtracted. Clearly, much more work is needed on this and other outstanding data reduction problems before an adequate image processing system is available for guest observers.

IUE observatory operations

The IUE observatory consists of the flight system plus the ground system. The flight system includes the spacecraft, the telescope, and the scientific instrumentation. The ground system includes the NASA IUE operations control centre at Greenbelt and the ESA's Villafranca satellite tracking station. The IUE observatory is designed to make maximum use of the continuous contact offered by the geosynchronous orbit. Guest observers are able to come to the observatory's science operations centres and actively participate in the real time control of their observations. In addition, the guest observer may take advantage of observing opportunities as they arise. Experienced resident astronomers and other observatory staff members assist the guest observer by providing advice on program planning, instrument operation, and data processing techniques. A guest observer usually arrives at the appropriate ground station a few days early to become familiar with the operations and data processing systems and to plan the final details of the observing program in consultation with resident astronomers.

The real time operations interface between the guest observer and the IUE is an interactive control and display terminal. This is manned by a telescope operator who is a specialist in spacecraft manoeuvring, target acquisition and instrument operation. The guest observer also sits at the display, and in consultation with the resident astronomer and telescope operator, directs critical aspects of the observation, including target acquisition, instrument operation, and data evaluation. The

interactive display provides the observer with all the information needed to plan slews, and also presents the field of view of the telescope to allow target identification. A quick-look spectrum is displayed as soon as the image is transmitted to the ground, and this allows the observer to evaluate the data quality and decide whether to repeat the observation or proceed to the next. The reduced data are available in the form of photographic reproductions of the raw and corrected images, verification plots of intensity versus wavelength, and magnetic data tapes. These materials are normally delivered to the observer 1 or 2 days after the observation.

Observers are given exclusive rights to their observations for 6 months after receipt of their data. The only exception to this rule is the right of an ESA observer to request full data on a duplicate target observed during a previous ESA shift. After 6 months, the data are deposited in the national space science data centre at Goddard Space Flight Center and in the data centres of the UK and the ESA. These data are available on request. Observers will be encouraged to use the data centres, rather than needlessly repeat observations; they may also find it advantageous to collaborate with other astronomers having similar observing programmes so that their combined observing time can be used to the greatest advantage.

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IUE observations of hot stars: HZ43, BD + 75°325, NGC6826, SS Cygni, η Carinae

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During the commissioning phase of IUE observations were made of a selection of hot stars: the white dwarf HZ43, the hot subdwarf BD + 75°325, the nucleus of the planetary nebula NGC6826, the dwarf nova SS Cygni, and the peculiar object η Carinae. The observations were made in the low dispersion mode and the data have been reduced using a preliminary calibration. The results are presented and discussed for each object.

THE IUE now provides the opportunity for major advances in the study of hot stars at UV wavelengths. The ability to detect faint sources now permits: (1) the study of hot, subluminescent stars which as a group represent the avenues to the endpoints of evolution for the great majority of stars; (2) the study of hot, massive stars in external galaxies (the Magellanic Clouds); and (3) the study of hot stars (and their surroundings) which are heavily reddened by the interstellar medium. Another basis for this opportunity is the provision for complete spectral coverage from 1,150 to 3,200 Å, which permits: (1) a detailed study of a

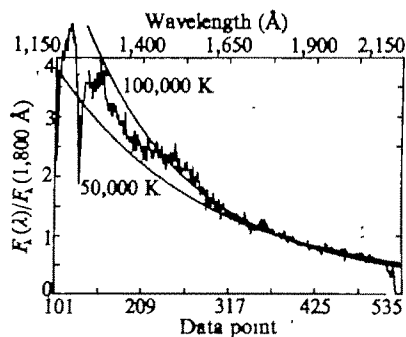


Fig. 1 Relative flux distribution of HZ43. Two abscissae are given, below, data point number of the spectrum extracted from the image; and above, the wavelength. The break in the spectrum near 1,230 Å is due to a rescan

spectral region usually inaccessible to Copernicus, roughly 1,450–1,800 Å, which contains several lines (for example, C IV λλ 1548, 1550; He II λ 1640; N IV λ 1719) which are very useful diagnostics of the physical conditions in hot stars; and (2) the study of flux distributions of hot stars over a wavelength interval wide enough to distinguish differences. Finally, the imaging capability of IUE yields an efficiency and, potentially, a freedom from time-dependent errors associated with scanning spectrometers. This capability permits programmes of study based on repeated observations of variable hot stars, such as the Ap stars, binaries and pulsating stars.

During the commissioning period of IUE a sample of hot stars were chosen for observation. The main criterion for selection was that they should represent classes of hot stars deserving thorough study but, by virtue of their faintness, were previously inaccessible to UV spectroscopy. Because of the short time allotted for these observations it was decided to concentrate on the hot, subluminescent stars but to try to sample as many types of this class as possible. In addition, the object η Car was selected because of its peculiarity and heavy reddening. All spectra were obtained at low dispersion and have a nominal resolution of 6 Å. Table 1 lists the stars observed.

The emphasis of the work described here was to assess the usefulness of IUE low-dispersion spectra to conduct studies of hot stars. However, hot stars were observed at high dispersion during the commissioning period as parts of investigations concerning X-ray sources¹ and the interstellar medium².

Observations and reductions

Table 2 lists some of the observational data: the object, the image number, date of observation (day number of year 1978), the entrance aperture (small or large), the exposure time and comments about the quality of the spectrum. Most of the latter are based on an inspection of photographic reconstructions of the raw spectral data which allow qualitative assessments of such characteristics as general exposure level, system focus, systematic noise, and effects of particle radiation. The exposure levels of most spectra seem to be near optimum. In the short wavelength spectrograph, the small decline in system sensitivity towards

shorter wavelengths is compensated for by the characteristic increase in flux of the hot, unreddened stars under study here. Hence, one well-exposed spectrum is useful for the full spectral range, from ~1,150 to 2,100 Å. The same generalisation holds true to a lesser extent for the long wavelength spectrograph covering the spectral region from 2,100 to 3,300 Å. The spectrograms generally show a low background level, and except for SWP 1141, which was obtained when the telescope was pointed near the Earth's limb, geocoronal Lyα is not present.

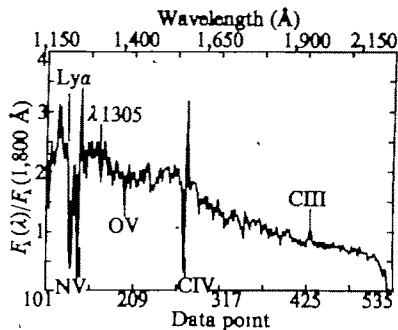


Fig. 3 Relative flux distribution of NGC6826

The spectral images were reduced by a process, still under development, described in the preceding article³. Any error in the zero-point of the wavelength scale was then removed by assigning the laboratory wavelength to an identifiable feature, such as Lyα. This procedure was possible for all spectrograms except for those of η Car where no features were readily identifiable. The net spectrum was converted to absolute flux units with the preliminary system sensitivity functions derived from standard star observations (described in ref. 3). This sensitivity function tends to produce a swell near 1,500 Å and it is possible that this is ultimately due to the OAO 2 calibration, which formed the basis for this preliminary calibration. When applied to the spectral region below 1,200 Å where the spectral response is dropping rapidly, this early calibration is much less accurate and the data below Lyα in the following figures should be treated with caution.

Table 1 Hot stars observed by IUE			
Object	Spectral type	V (mag)	Notes
BD+75°325	sdO	9.5	
HZ43	DAwk	12.8	EUV source
NGC6826	O3f	10.5	Central star of planetary nebula
SS Cyg	G+em lines	9.1*	Dwarf nova
		11.0*	
η Car	pec	6.2	UV-IR object

* Magnitude estimated from FES counts at time of observation.

HZ43

The white dwarf HZ43 (spectral type DAwk) has long been known to be one of the hottest objects from ground-based observations^{4,5}. In recent years, it has been an object of considerable interest because it was the first extra-solar source of EUV radiation to be detected^{6,7}. The IUE spectrum of HZ43, shown in Fig. 1, shows no obvious spectral features other than Lyα. This is consistent with the interpretation that HZ43 is a white dwarf in which gravitational separation has already taken place so that hydrogen would be the only observable element in the stellar atmosphere. The Lyα feature observed here is probably essentially stellar rather than interstellar in origin, as the star is close to the Earth (about 60 pc)^{8,9} and the gravity of the star is high (log g > 7.7) (ref. 10). Although Lyα is the only prominent feature, there appears to be 'fine structure' in the spectrum near 1,720 Å, which if confirmed could be ascribed to N IV.

Fig. 2 Relative flux distribution of BD+75°325

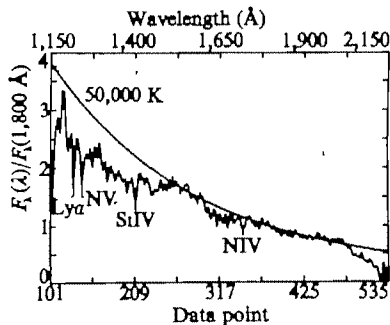


Table 2 Low dispersion spectral images obtained by IUE

Object	Image*	Date (day 1978)	Start (GMT)	Aperture	Exposure time (s)	Comments
BD + 75°325	SWR 1024	41	14.27	S	600	Camera noise
	LWP 1024	41	19.40	S	210	
HZ43	SWR 1057	57	07.51	L	1,200	Reoptimised camera parameters used
	SWR 1058	57	11.50	S	360	
SS Cyg			12.14	L	180	Reoptimised camera parameters used
	LWR 1138	72	06.08	L	170	
	SWR 1050	57	15.51	S	150	Geocoronal Ly α present
			16.23	L	168	
η Car	SWP 1141	72	22.42	L	480	Reoptimised camera parameters used
	SWR 1060	57	00.16	L	150	Zero-point of wavelength scale uncertain
	LWR 1026	58	23.12	L	15	Zero-point of wavelength scale uncertain

* Images are designated by camera and sequence number. SWR=short-wavelength redundant camera; LWP=long-wavelength prime camera, SWP=short-wavelength prime camera, LWR=long-wavelength redundant camera

The IUE flux distribution indicates a black body temperature between 50,000 and 100,000 K with negligible interstellar extinction, values that will be improved when the system is fully optimised and calibrated. Auer and Shipman¹⁰ from EUV and visual data estimate that the stellar parameters lie between the following limits: $T_{\text{eff}} = 55,000$ K, $\log g = 7.7$, $M = 0.4M_{\odot}$ and $T_{\text{eff}} = 70,000$ K, $\log g = 7.9$, $M = 0.5M_{\odot}$.

BD + 75°325

At a visual magnitude of 9.5, BD + 75°325 (spectral type sdO) is one of the brightest hot subdwarfs in the sky and has been studied in detail by Peterson¹¹. The only prominent lines in the visual spectrum are due to hydrogen, helium and nitrogen. The IUE spectrograms show a similar picture: absorption lines of He II, N IV and N V are strong, while lines of C III and C IV are below the threshold of detection. The long wavelength spectrum (not shown) indicates that the Paschen series of He II is stronger than in young O stars like ζ Pup¹², probably due both to helium enrichment and to Stark-broadening. The short-wavelength spectrum, shown in Fig. 2, indicates that nitrogen lines are strong, totally in absorption, and apparently at their rest wavelengths. The absorption in the spectral region, 1,600–1,750 Å, is real and most probably includes He II $\lambda 1640$. There is no evidence for the presence of the C IV resonance doublet at 1,550 Å. The apparent emission feature shortwards of Ly α is a noise spike.

In her study of the visual spectrum of BD + 75°325, Peterson¹¹ found that it was impossible to construct an LTE model atmosphere which predicts the observed characteristics of the star: the ionisation equilibrium between N III and N IV implies the atmospheric parameters, $T_{\text{eff}} = 55,000$ K, $\log g = 5.3$ and $N_{\text{He}}/N_{\text{tot}} = 0.6$, while the helium lines imply the parameters $T_{\text{eff}} = 45,000$ K, $\log g = 5.3$ and $N_{\text{He}}/N_{\text{tot}} = 0.6$. It is probable that much of the discrepancy is due to non-LTE effects. If so, it is

Fig. 4 Comparison of IUE spectrum of NGC6826 with Copernicus spectra of ζ Pup and o Per. The Copernicus spectra have been smoothed by a running box average whose width corresponds roughly to 6 Å.

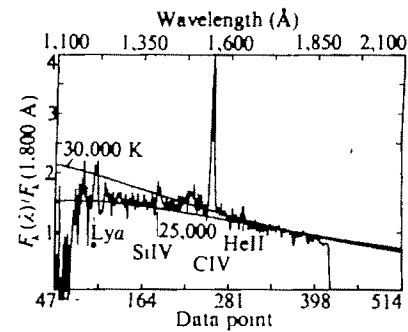
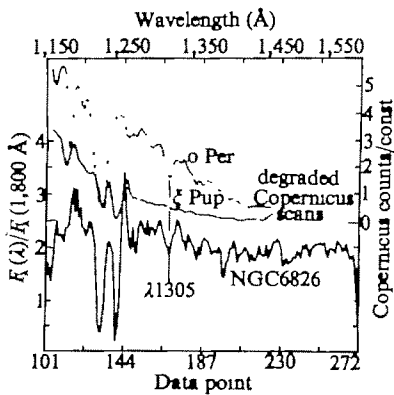


Fig. 5 Relative flux distribution of SS Cyg near quiescence

important to obtain line strengths and profiles of important transitions of helium and nitrogen. High-dispersion spectra of BD + 75°325 could be obtained easily by IUE and should provide useful constraints on the physical properties and chemical abundances in the stellar atmosphere

NGC6826

Figure 3 shows the short wavelength spectrum of NGC6826. The spectrum of the central star of this planetary nebula clearly has a high excitation, as would be expected from its visual spectral type of O3f (ref. 13). The most prominent lines are the resonance lines of N V $\lambda 1240$ and C IV $\lambda 1550$, as well as O V $\lambda 1370$ and N IV $\lambda 1718$ which arise from excited levels. The UV spectrum is very similar to that of the young star ζ Pup¹⁴, also an early Of star, with the strong lines all having P-Cygni profiles which indicate mass-loss from the central star. The interaction of the stellar wind with the surrounding nebula would be an interesting topic to pursue.

Although the stellar contribution is dominant, the spectrum of this object is a composite of stellar, nebular and interstellar spectra. Nebular emission of C III $\lambda 1909$ is clearly visible and fills the large aperture ($10'' \times 20''$). Nebular and/or interstellar absorption is also present: the strongest such feature, at 1,305 Å, is undoubtedly a blend of O I, Si II and P II resonance lines, all of which are of too low ionisation to belong to the stellar atmosphere. Figure 4 compares the spectral region containing this feature with degraded Copernicus U2 spectra of ζ Pup, which has very little reddening ($E(B - V) = 0.03$), and o Per, a B1 III star with heavy reddening ($E(B - V) = 0.32$). Note that the $\lambda 1305$ feature does not appear in ζ Pup but does appear in the spectrum of o Per with a strength comparable with that in the spectrum of NGC6826. If the relation between hydrogen column density and reddening given by Bohlin¹⁵ is used with the colour excess derived from Kaler¹⁶, a value of $\sim 10^{20} \text{ cm}^{-2}$ is obtained which is rather low for the observed line strength. There is therefore a possibility that some of the 1,305 Å feature may be nebular in origin but because of the loose nature of the correlation between hydrogen column density and reddening,

this would need to be established by other evidence. If the presence of nebula absorption lines were confirmed, for example, by high dispersion spectra, it would provide an important new tool for abundance analysis.

SS Cygni

The properties of the dwarf nova, SS Cyg, and dwarf novae in general have been reviewed by Warner¹⁷; since when, it was discovered¹⁸ and confirmed¹⁹ that SS Cyg is a soft X-ray source. The general explanation for these properties is that dwarf novae are binary systems in which material is being transferred from a low-mass secondary, through an accretion disk, to a white-dwarf companion. The 'hot spot', where the stream of transferred matter collides with the disk, and the disk as a whole are responsible for the UV and visual radiation.

UV spectra of SS Cyg were obtained on two occasions during the commissioning phase of IUE: the first during outburst, and the second near quiescence. The two spectra, which are shown in Figs 5 and 6, show the effect of an outburst. The most outstanding change is an increase in the continuous flux at outburst causing a reversal in the appearance of the CIV $\lambda 1550$ resonance doublet: near quiescence, it is an exceedingly strong emission feature, while during outburst it is a weak absorption or P-Cygni feature.²⁰ In addition, emission at NV $\lambda 1240$, Si IV $\lambda 1400$ and He II $\lambda 1640$ are visible near quiescence, while during outburst these lines either disappear or go into absorption. The great strength of CIV emission near quiescence is important as an indicator of physical conditions in the disk.

A second change is an apparent increase in colour temperature during the outburst as expected from UV photometry of SS Cyg by Holm and Gallagher²⁰. The IUE spectra are similar to a black body temperature near 40,000 K during outburst maximum, and about 30,000 K near quiescence. As the interstellar extinction of this object is minimal ($E(B-V) \approx 0.08$) (ref. 20) these estimates should only slightly underestimate the intrinsic black-body colour temperatures of SS Cyg in this wavelength range. Of course, these values should be treated with caution because they are highly dependent on the absolute calibration, and they are not directly translatable to physical temperature until the mechanism of emission (probably optically thick free-free emission) and the range in physical conditions within the disk are specified.

η Car

One of the most remarkable objects in the sky is the star (or starlike object), η Car. Listed as a 4th mag star in Bayer's atlas of 1603, it brightened in the early 1800s until in 1843, it outshone every star in the sky except Sirius. For the past century, it has hovered between about 6th and 8th mag. The evolutionary status of this object is not understood, and theories about its nature²¹ are diverse, ranging from the hypothesis that it is a young, massive star either approaching the main sequence or somewhat evolved from it, to the hypothesis that it is a non-thermal or non-stellar object, such as a supernova remnant. Its present spectrum²²⁻²⁴ in the visual, consists of a continuum on which are superposed numerous emission lines (both permitted and forbidden) of Fe II, Cr II, Ni II, Na I, and so on, as well as the

Fig. 6 Relative flux distribution of SS Cyg during outburst.

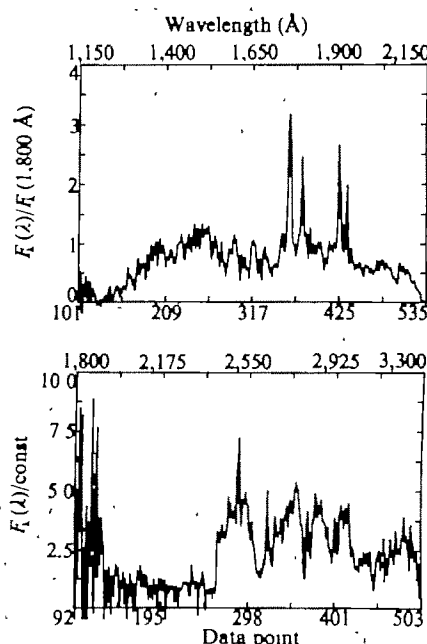
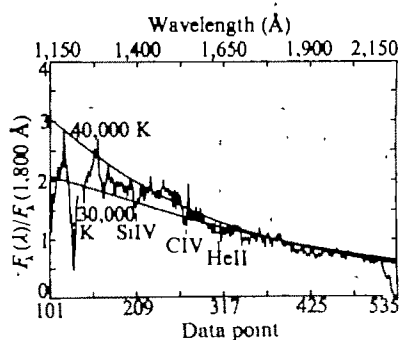


Fig. 7 Relative flux distributions of η Car

hydrogen Balmer series. According to Rogers and Searle²⁴, the continuum contributes 70% and the emission lines 30% of the visual radiation. The object is heavily reddened both by interstellar material in the line of sight and by circumstellar gas and dust.

The IUE spectra of η Car, shown in Fig. 7, indicate that the UV spectrum is very complicated. One of the most outstanding characteristics is that the spectrum appears to change from an emission-line-dominated spectrum at longer wavelengths to an absorption-line-dominated spectrum at shorter wavelengths. According to Rogers and Searle, there are only a few spectral windows in the visual region of the spectrum which are free of emission lines, and in these regions the continuum is featureless. However, in the far-UV region from 1,150 to 1,600 Å, there appear to be about 30 absorption features visible, but it is not clear at this stage whether these are real or whether they are simply regions where there are no emission lines.

Another unusual characteristic of the UV spectrum is a flux discontinuity, near 2,400 Å. Although interstellar extinction must play a part in depressing the flux in this spectral region, the drop is too precipitous to be ascribed totally to the interstellar $\lambda 2200$ extinction bump. Possibly it is due to a chance lack of emission lines below 2,400 Å. In any case, the discontinuity greatly increases the difficulty of inferring the amount of extinction from the observed UV flux distribution.

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IUE observations of cool stars: α Aurigae, HR1099, λ Andromedae, and ϵ Eridani

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Initial IUE observations of four cool stars are reported. Observed fluxes and surface fluxes are given for several UV emission lines in the spectral range 1,175–2,000 Å, obtained at low and high dispersion with the short-wavelength spectrograph and camera. These lines are formed in the outer atmospheres of these stars, in regions presumably analogous to the solar chromosphere and transition region. The surface fluxes in the lines increase along the sequence: quiet Sun, ϵ Eri, λ And, α Aur, and HR1099. The 2.8-d RS CVn-type binary HR1099, observed on 1 March 1978 near the end of a major flaring episode, has line surface fluxes ~ 100 times that of the quiet Sun, similar to those seen in solar flares. Line profiles and flux ratios in multiplets for Capella are presented, and comments given on the opacity of the lines and on a tendency of line width to increase with temperature of formation.

THE IUE has made it feasible to study for the first time UV spectra of many late-type stars. These emission-line spectra originate in chromospheres, coronae, and circumstellar envelopes, and they contain information about mass loss from late-type stars. Before IUE, Copernicus and rocket- and balloon-borne experiments could study only the strongest lines in a few very bright stars. Here we present observations of α Aur (Capella), λ And, HR1099, and ϵ Eri. These data were obtained during the science commissioning phase of IUE and Table 1 gives the circumstances of the observations. Although these data were obtained before reoptimisation of the telescope and detectors, they indicate the ultimate capabilities of IUE. In this first sampling of cool stars, we have chosen those expected to have bright emission-line spectra and have concentrated on the 1,175–2,000 Å region, where most of the important emission lines are located.

α Aurigae (Capella)

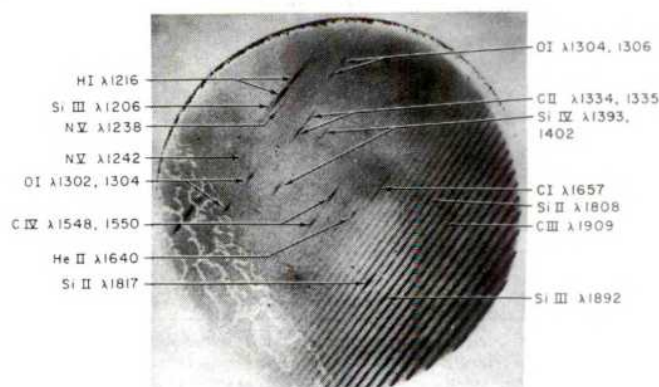
Capella is a double-line spectroscopic binary system, consisting of a G5 III primary (Capella A) and a G0 III secondary (Capella B)¹. It was chosen as the first IUE scientific target because it is easily identifiable and is known to have several bright UV emission lines.

Evidence for a chromosphere in at least one component consists of emission cores in the Ca II H and K lines², the Mg II h

($\lambda 2803$) and k ($\lambda 2796$) lines³, and H I Ly α ^{4,5}. Using the Ca II and Mg II line fluxes and identifying the chromospheric spectrum with Capella A^{3,4}, Kelch *et al.*² derived a model for the chromosphere and upper portion of the photosphere of this star. Material at temperatures above 20,000 K is indicated by UV emission lines of C II–IV, Si II–IV, N V, and O VI^{4–6}. Haisch and Linsky⁷ have interpreted these data in terms of a geometrically narrow conductively heated transition region similar to the transition region in a solar plage. Even hotter material is indicated by X-ray observations^{8,9} and possibly by the observed strong He I $\lambda 10830$ absorption¹⁰. The very recent HEAO-1 observations of Cash *et al.*¹¹ are consistent with a 10^7 K isothermal plasma with solar composition.

In the two exposures obtained of Capella on 9 February 1978, the large aperture ($10'' \times 20''$) included nearly all of the slightly out-of-focus stellar image. The first image was a high-dispersion exposure of the long-wavelength spectrum (1,900–3,200 Å) with the prime camera (image LWP 1022). This spectrum shows the Mg II $\lambda 2796$, 2803 lines in emission and the photospheric absorption line spectrum well exposed for 2,530–2,880 Å and 3,100–3,270 Å, but largely overexposed for 2,880–3,100 Å. The second observation was a high-dispersion exposure of the short-wavelength spectrum (1,175–2,100 Å) with the redundant camera (image SWR 1022). Qualitatively this consists of emission lines shortwards of 1,600 Å, and a photospheric absorption-line spectrum with superimposed emission lines at longer wavelengths. This spectrum is shown in Fig. 1 with the

Fig. 1 Short-wavelength high-dispersion raw spectrum of Capella (image SWR 1022) in the IUE echelle format. Important emission lines are identified.



important emission lines identified. Table 2 lists measured wavelengths of emission lines or blends and the spectrum orders. Also included are proposed identifications giving ion, multiplet number, and laboratory wavelength based on the solar line lists of Burton and Ridgeley¹² and Doschek *et al.*¹³ The relative wavelength scale was obtained from a previous Pt-Ne lamp exposure, but with 140.3 km s⁻¹ added to fit optimally the measured wavelengths of the 13 bright single narrow emission lines. This correction allows for errors in the grating dispersion constants and for the stellar radial velocity and heliocentric motions, but it prevents a velocity measurement of the emission line spectrum relative to the mean velocity of the binary system. The measured wavelengths of all bright single identified lines agree with the laboratory wavelengths to within 0.05 Å.

Relative line fluxes were determined by subtracting the background and the stellar continuum if present, and then integrating the net signal across the emission lines. These fluxes, in IUE flux units, are given in Table 2 for α Aur and for a preceding 45-s exposure of the standard star η UMa obtained with similar observing parameters. Where stellar or interstellar absorption lines are present in the η UMa spectrum, we interpolated between continuum regions on either side. Dividing the two spectra provides the best means of estimating fluxes at Earth for Capella, corrected for the echelle blaze characteristics and variable detector sensitivity with position. We have assumed the η UMa absolute fluxes of Code and Meade¹⁴, again interpolating between absorption features where necessary shortwards of 1,400 Å.

Table 1 Summary of observations

Star	Spectral type	<i>w</i> v	Binary period (d)	Phase (d)	Image no.	Dispersion	Exposure midpoint (JD 244+)	Exposure (min)	Comments
α Aur (Capella)	G5 m+GO m	0.09	104.023	77.670	LWP 1022	High	3549 321	3	
				77 791	SWR 1022	High	3549 442	70	
λ And	G8 m-rv	3.88	20 5212	13.438	SWR 1044	High	3560 146	240	Underexposed
				13.715	SWR 1045	Low	3560.423	152	
HR1099 ε Eri	G5 v [†] K2-v	6.0 3 73	2.83782 —	2.4488	SWR 1065	Low	3568.783	100	
					SWR 1067	Low	3569.383	38	Underexposed
					SWR 1069	Low	3569.619	140	

Table 2 Emission lines observed in Capella

Measured wavelength (Å)	Spectral order	Identification ion	λ (Å)	FWHM (Å)	IUE flux		Flux at Earth (erg cm ⁻² s ⁻¹)	Surface flux	Surface flux (α Aur/quiet Sun)	Comments
					α Aur	η UMa*				
1,175.26	117	C m(4)	1,175†		8.0(2)‡	7.2(4)‡				Very uncertain Uncertain due to Lyα spillover from adjacent order
1,206.45‡	114	Si m(2)	1,206 51		1 5(4)‡	8 0(4)‡	2.0(-11)‡	6 6(4)‡	20‡	
1,215.58‡	113	H r(1)	1,215 66	1 51	2.9(5)					
	113	O v	1,218 39							Not apparent
1,237.28‡	111	?			5.4(3)‡	2.7(5)	2.9(-12)‡	9.4(3)‡		Uncertain
1,238.70‡	111	N v(1)	1,238 82	0 75	1 0(4)‡	2.9(5)	5.0(-12)‡	1 6(4)‡	32‡	Not apparent
	111	[Fe xii]	1,242 03							
1,242.80	111	N v(1)	1,242.80	0 67	3.8(3)‡	4.4(5)	1.3(-12)‡	4.1(3)‡	12‡	
1,302.14	106	O i(2)	1,302 17	0.50	1.6(4)	4.2(5)	6 5(-12)	2.1(4)	17	
1,304.83	106	O i(2)	1,304.86	0.55	1 6(4)	3.2(5)	8 2(-12)	2 7(4)		
1,304 85	105	O i(2)	1,304.96	0.48	1 1(4)	1.8(5)	1.0(-11)	3.4(4)	20	Near edge of order
1,305.98	106	O i(2)	1,306 03	0 58‡	1.2(4)	2.7(5)	7.7(-12)‡	2.5(4)‡		Obscured by blemish
1,306.05	105	O i(2)	1,306 03	0.41	1 3(4)	2.5(5)	8.6(-12)	2.8(4)		
1,334.55	103	C ii(1)	1,334.53	0.85	3 2(4)	4.0(5)	1.3(-11)	4.2(4)	20	
1,335.69	103	C ii(1)	1,335.66	0 78	4.0(4)	4.4(5)	1 5(-11)	4.8(4)		
	103	[Fe xii]	1,349.38							Not apparent
1,355.50	102	O i(1)	1,355.60	0.36‡	3 4(3)‡	3.2(5)	1.7(-12)‡	5 5(3)‡	17‡	Present but weak
	102	O i(1)	1,358.51							
1,393.75	99	Si rv(1)	1,393.76	0.63	2 2(4)	3 5(5)	9.2(-12)	3.0(4)	17	
1,401.37‡	98	O rv	1,401 16	0 96‡	1.3(4)‡	2.9(5)	6.4(-12)‡	2.1(4)‡	160‡	
1,402 76	98	Si rv(1)	1,402.77	0 71	2.0(4)	3.1(5)	9.5(-12)	3 1(4)	47	
1,404.6‡	98	O rv	1,404 81			3.5(5)				Possibly present
1,548.20	89	C iv(1)	1,548 20	1.02	4.1(4)	1.9(5)	2.8(-11)	9.3(4)	25	
1,550 80	89	C iv(1)	1,550.77	0 75	2.0(4)	2 0(5)	1.3(-11)	4 4(4)	21	
1,560.88	88	C i(3)	1,560 61	0 24‡	7 6(2)	1.4(5)	7.0(-13)	2 3(3)	4 5	
1 561.38	88	C i(3)	1,561.4†	0 38‡	2 3(3)	1.5(5)	2 0(-12)	6.6(3)		
1,563 52	88	Fe iii(45)	1,563.79			1 7(5)				Possibly present
1,640.47	84	He ii(12)	1,640 41	0 53	1 5(4)	2 0(5)	7.9(-12)	2 6(4)	20	Additional features at 1639.93 (possibly due to Fe ii 1640.15) and 1640 98
1,657.15‡	83	C i(2)	1,657.1†	2.17	2.3(4)	2.1(5)	1.1(-11)	3.7(4)	7.0	Peaks at 1656 33 (due to C i 1656.27) and at 1657.80 (due to C i 1657 91)
1,808.05	76	Si ii(1)	1,808.01	0.42	3 3(4)	3.5(5)	8 1(-12)	2.7(4)	4.0	
1,816.93	76	Si ii(1)	1,816.93	0 43						
1,817.3‡	76	Si ii(1)	1,817 45		1.0(5)	3.5(5)	2.5(-11)	8.3(4)	8.3	
1,892 00	73	Si m(1)	1,892 03	0.69	8 4(4)	3 3(5)	1 9(-11)	6.2(4)	‡	
1,908 71	72	C iii	1,908 73	0.54	3.5(4)	3 9(5)	6.5(-12)	2 1(4)	‡	
1,992 88	69	?		0 32	1.1(4)	3 9(5)	1 7(-12)	5 6(3)	‡	
1,993.59	69	C i(32)	1,993 62	0.28	1 6(4)	4.1(5)	2.4(-12)	7.9(3)	‡	

* Per Å interpolated to line centre if necessary to avoid absorption lines
† Multiplet, wavelength given is for the mean of the solar emission feature.
‡ Line not in emission in spatially-averaged solar spectrum.
‡ Uncertain.

For stars with a bright continuum like η UMa, inter-order light is not a good measurement of the background because it includes the spillover of light from adjacent orders, especially in the short-wavelength portion of the spectral format, where the orders are most closely spaced. We have therefore determined the background for η UMa as a linear function of position perpendicular to the echelle dispersion direction such that the flux in the centre of the interstellar Ly α line is zero and the background equals the inter-order signal between the two longest-wavelength orders, which are the most widely separated.

Surface fluxes (fluxes per unit area at the stellar surface) for Capella are derived from the measured fluxes at Earth and an angular diameter for Capella A of 0.0072" (ref. 2). These surface fluxes were then divided by the corresponding quiet Sun disk average values of Rottman (personal communication), which were obtained near solar minimum.

Inspection of unsmoothed line profiles is a good way of assessing the quality of the IUE observations of cool stars. The Ly α line shown in Fig. 2 is very well exposed, with signal-to-noise ratio ~ 30 near the peak. However, the interstellar hydrogen absorption feature does not reach zero intensity at line

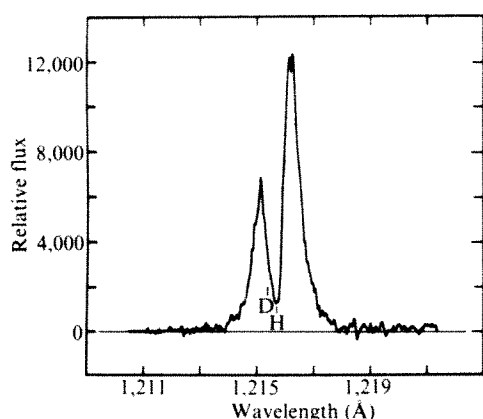


Fig. 2 Reduced profile of the Capella Ly α line in relative IUE flux units. Location of interstellar features due to hydrogen and deuterium Ly α is noted.

centre and interstellar deuterium absorption barely appears, indicating a degraded spectral resolution due to telescope mis-focus at the time of this early observation.

Weaker lines permit measurement of line fluxes and widths, but not details of the line profiles. The C IV lines in Fig. 3 have signal-to-noise ratio ~ 8 near their peaks and an integrated line flux ratio of 2.1, compared to the solar ratio of 1.8. The O I resonance lines shown in Fig. 4 have a similar signal-to-noise ratio to the C IV lines, except for the $\lambda 1306$ line which is located near the end of order 106. Finally the C II lines shown in Fig. 5 have peak signal-to-noise of perhaps 6, yet the line fluxes and line flux ratio can be fairly accurately measured as there are about 220 pixels per line.

ϵ Eridani

This bright K2 V star was observed as a prototype late-type dwarf and also as a single star against which to compare the binary systems Capella, λ And, and HR1099. Emission in the cores of the Ca II and Mg II resonance lines had earlier provided evidence for a chromosphere in this star. From measured surface fluxes 2.3 and 1.6 times those in the average quiet Sun for Ca II and Mg II, respectively, Kelch¹⁵ derived a chromospheric model.

We obtained two low-dispersion short-wavelength observations (see Table 1), but here discuss only the well-exposed SWR 1069 image. This spectrum was calibrated using the preliminary sensitivity curves for this camera¹⁶, and is compared with the quiet Sun spectrum of Rottman (personal communication) degraded to the low-dispersion resolution in Fig. 6.

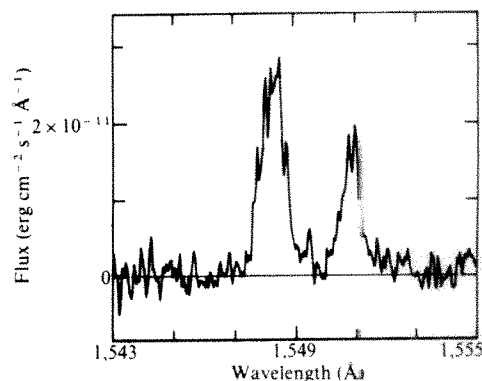


Fig. 3 Reduced profiles of the Capella C IV $\lambda\lambda 1548, 1550$ lines in absolute flux units.

Except for the relative weakness of the continuum at long wavelengths, which is expected for the cooler star, the spectrum of ϵ Eri is qualitatively similar to that of the Sun. The broad, saturated feature which covers the stellar Ly α and Si III $\lambda 1206$ lines is due to geocoronal Ly α emission filling the 10" $\times$ 20" large aperture.

Table 3 lists the emission features that clearly show up above the noise, their identification, and the fluxes at Earth. Only those features which appear in two or more stars are included. For analytical purposes, the most interesting quantities are the surface fluxes, which we determine from an angular diameter of 0.00229" derived by the V-R relation of Barnes and Evans¹⁷. The surface fluxes for ϵ Eri and for the average quiet Sun are listed in Table 3.

λ Andromedae

λ And is a single-lined spectroscopic binary with a period of 20.5 d and is a long-period RS CVn-type binary variable. This star shows bright and variable emission cores in the Ca II (ref. 18), Mg II, and Ly α lines which may be a consequence of the primary filling a significant fraction of its Roche lobe¹⁹. We obtained both high- and low-dispersion short-wavelength images of λ And. The high-dispersion image is underexposed, but it weakly exhibits emission in the following lines: Ly α ; O I $\lambda\lambda 1302, 1304, 1306$; C II $\lambda\lambda 1334, 1335$; C IV $\lambda\lambda 1548, 1550$; He II $\lambda 1640$; Si II $\lambda\lambda 1808, 1817$; and Si III $\lambda 1892$. As a result of the faintness of this exposure, we observed the star again but in low dispersion. This spectrum was calibrated in a manner similar to that of ϵ Eri and is shown in Fig. 6. Table 3 gives line fluxes and surface fluxes, for which an angular diameter of 0.00274" was assumed.

The low-dispersion spectrum was obtained at spectroscopic phase 13.751 d with respect to the orbital elements of Walker²⁰. λ And has a photometric period of 50–55 d. In unpublished UBV photometry Hall found that λ And was brightest on JD 2,443,360 and 2,443,420. Thus our observations were obtained very near photometric maximum, when IUE was observing the unspotted and presumably least active hemisphere.

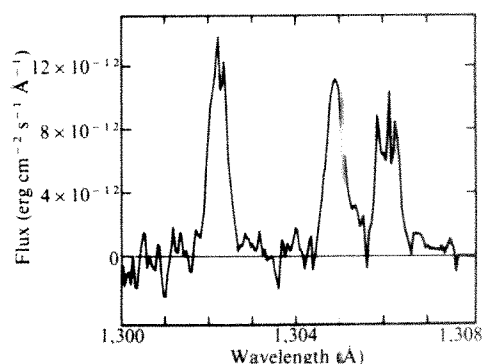


Fig. 4 Reduced profiles of the Capella O I $\lambda\lambda 1302, 1304, 1306$ lines in absolute flux units.

Table 3 Emission lines observed at low dispersion

Wavelength (Å)	Identification	Flux at Earth (erg cm ⁻² s ⁻¹)			Surface flux (erg cm ⁻² s ⁻¹)			
		HR1099	λ And	ϵ Eri	HR1099	λ And	ϵ Eri	Sun
1,175	C II(4)	4.4(-13)	3.0(-13)	—	1.8(5)	8.4(3)	—	1.6(3)
1,215	H I	SAT	SAT	SAT	—	—	—	2.1(5)
1,240	N V(1)+[Fe XII] ⁹	1.4(-13)	1.9(-13)	1.4(-13)*	5.7(4)	5.3(3)	4.5(3)*	8.5(2)
1,263	Si II(4)	4.3(-14)*	2.5(-14)*	2.1(-13)*	1.8(4)*	7.0(2)*	6.8(3)*	1.1(3)
1,276	C I b1	2.4(-14)*	3.2(-14)*	9.9(-14)*	9.8(3)*	8.9(2)*	3.2(3)*	5.2(2)
1,281	C I(5)	6.4(-14)	—	1.2(-13)*	2.6(4)	—	3.9(3)*	—
1,304	O I(2)	6.0(-13)	2.1(-12)	4.6(-13)	2.5(5)	5.9(4)	1.5(4)	4.0(3)
1,335	C II(1)	9.0(-13)	6.7(-13)	4.7(-13)	3.7(5)	1.9(4)	1.5(4)	4.6(3)
1,357	O I(1)	6.7(-14)	2.5(-13)	—	2.7(4)	7.0(3)	—	3.4(2)
1,371	O V(7)	4.0(-14)	3.1(-14)*	—	1.6(4)	8.6(2)*	—	—
1,382	Fe II+Al III	2.1(-14)*	3.2(-14)*	—	8.6(3)*	8.9(2)*	—	—
1,394	Si IV(1)	2.3(-13)	3.2(-13)	1.5(-13)	9.4(4)	8.9(3)	4.9(3)	1.7(3)
1,403	Si IV(1)+O IV	2.3(-13)	5.0(-13)	1.8(-13)	9.4(4)	1.4(4)	5.8(3)	7.8(2)
1,425	Fe II(47)+Si(5)	2.4(-14)*	1.2(-13)	1.3(-13)*	9.8(3)*	3.3(3)	4.2(3)*	—
1,433	C I(65)+Si(5)	6.0(-14)	3.1(-14)*	9.0(-14)*	2.5(4)	8.6(2)*	2.9(3)*	2.1(2)
1,470	C I(36)+Si(4)	5.8(-14)	8.8(-14)	1.7(-13)*	2.4(4)	2.5(3)	5.5(3)*	5.2(2)
1,482	Si(4)+Si(3)	6.3(-14)	—	1.5(-13)*	2.6(4)	—	4.9(3)*	5.3(2)
1,530	Si II(2)	1.5(-13)	4.7(-14)	1.9(-13)*	6.2(4)	1.3(3)	6.2(3)*	2.3(2)
1,549	C IV(1)	1.3(-12)	1.8(-12)	5.3(-13)	5.3(5)	5.0(4)	1.7(4)	5.8(3)
1,561	C I(3)+Fe II(45)	1.4(-13)	—	3.7(-13)*	5.7(4)	—	1.2(4)*	2.0(3)
1,640	He II(12)+Fe II(43)	6.0(-13)	1.0(-12)	4.9(-13)	2.5(5)	2.8(4)	1.6(4)	1.3(3)
1,657	C I(2)+Fe II	3.6(-13)	5.9(-13)	3.6(-13)	1.5(5)	1.6(4)	1.2(4)	5.3(3)
1,670	Al II(2)+O III+Fe II	8.4(-14)*	3.3(-13)	8.8(-14)*	3.4(4)*	9.2(3)	2.9(3)*	1.5(3)
1,686	Fe II(40)	4.4(-14)	5.2(-14)*	6.7(-14)*	1.8(4)	1.5(3)*	2.2(2)*	—
1,696	Fe II(38)	7.7(-14)	4.5(-14)	1.7(-13)	3.2(4)	1.3(3)	5.5(3)	—
1,714	Fe II b1	5.2(-14)	5.9(-14)	2.9(-14)*	2.1(4)	1.6(3)	9.4(2)*	—
1,725	Fe II b1	5.2(-14)	4.9(-14)*	8.7(-14)*	2.1(4)	1.4(3)*	2.8(3)*	—
1,748	Ni II(5)	6.9(-14)	8.1(-14)	—	2.8(4)	2.3(3)	—	—
1,763	Fe II(101)+C I	—	2.8(-14)*	8.8(-14)*	—	7.8(2)*	2.9(3)*	—
1,788	Fe II	4.7(-14)	8.5(-14)	—	1.9(4)	2.4(3)	—	—
1,808	Si II(1)	2.1(-13)	7.1(-13)	3.3(-13)	8.6(4)	2.0(4)	1.1(4)	6.1(3)
1,817	Si II(1)	5.1(-13)	SAT	9.4(-13)	2.1(5)	—	3.1(4)	1.0(4)
1,842	Fe II(65)	2.8(-14)	3.2(-14)	—	1.1(4)	8.9(2)	—	—
1,855	Al III(1)+Fe III(63)	8.1(-14)	9.7(-14)	—	3.3(4)	2.7(3)	—	—
1,863	Al III(1)+Fe II b1	7.4(-14)	1.1(-13)	—	3.0(4)	3.1(3)	—	—
1,892	Si III(1)	1.5(-13)	2.5(-13)	7.2(-14)	6.2(4)	7.0(3)	2.3(3)	—
1,900	Si(1)+Fe	8.9(-14)*	1.9(-13)	1.2(-13)	3.7(4)	5.3(3)	3.9(3)	—
1,909	C III	1.7(-13)	1.3(-13)	—	7.0(4)	3.6(3)	—	—
1,931	C I(33)	7.2(-14)	7.5(-14)	9.6(-14)	3.0(4)	2.1(3)	3.1(3)	—
1,948	Fe II(123)	8.4(-14)*	2.8(-14)	1.2(-13)	3.4(4)	7.8(2)	3.9(3)	—
1,993	C I(32)+Fe III(81)	2.4(-14)	1.7(-13)	6.6(-14)	9.8(3)	4.7(3)	2.1(3)	—

* Uncertain

HR1099

Bopp and Fekel²¹ pointed out that HR1099 is a double-lined spectroscopic binary, and is one of the shortest-period RS CVn-type binaries. They show that the Ca II H and K and H α emissions are due mainly to the primary star. HR1099 also shows Ly α , Mg II h and k, radio, and X-ray emission²²⁻²⁴. We observed this object on 1 March 1978, towards the end of the most intense flaring episode yet observed for this system²⁵. The observed spectrum of HR1099 is shown in Fig. 6 and fluxes are given in Table 3. From the G5 V spectral type²⁶, we derived $V-R=0.61$ and hence an angular diameter of $0.000644''$. Our observation occurred 2.4488 d after conjunction²⁷, when the primary, and presumably more active, component was near maximum velocity of approach²¹.

Discussion

Detailed analysis and atmospheric modelling of these data will be reported elsewhere, and both deeper exposures and observations at different phases of the binary targets would be helpful. In the meantime it is important to discuss and assess these new data qualitatively.

First, there is no clear evidence of 10^6 K or hotter material in the 1,175–2,000 Å spectral region, but as only very weak coronal lines such as [Fe XII] $\lambda\lambda$ 1242.03, 1349.38 occur in this spectral range, this conclusion is not unexpected. These lines do not appear in the quiet Sun disk spectrum but they do appear off the limb above active regions and during flares²⁸. The hottest plasma clearly identified in our data has a temperature of 2.6×10^5 K, as indicated by the O V λ 1371 line in HR1099 and λ And. However, we point out that the broad feature at 1,355 Å in HR1099 and ascribed to O I(1) λ 1355.6 and λ 1358.5 could

include a contribution due to [Fe XXI] λ 1354.1, which is bright in solar flares²⁹.

Second, there are important qualitative differences among the five spectra shown in this paper (Figs 1 and 6). In both the Sun and Capella the photospheric absorption-line spectrum is clearly present at wavelengths longwards of 1,500–1,600 Å, but is very weak in the cooler stars. It is likely that Capella B is mainly responsible for the observed photospheric spectrum of Capella.

Line ratios often vary strongly from star to star. To facilitate comparison, Table 4 lists the ratios of accurately measured line surface fluxes to corresponding values for the average quiet Sun. Average values are given for ions whose lines are formed in similar temperature ranges³⁰, and we have included Ly α , Ca II, and Mg II data from other sources.

There are several trends in these data. First, the O I resonance lines are often much brighter than the other chromospheric ($T < 10^4$ K) lines, and the He II λ 1640 line is often much brighter than the other transition-region ($T > 3 \times 10^4$ K) lines. The O I anomaly is probably due to the Ly β pumping mechanism that operates in α Boo³¹ and the Sun³², and the He II λ 1640 line can be formed by recombination following photo-ionisation of He II by $\lambda < 227$ Å coronal photons³³. Thus, when the O I and He II lines seem anomalously bright, one may infer that these fluorescent processes are more important compared to collisional excitation than in the Sun.

In ϵ Eri the chromospheric and transition-region lines, except for He II λ 1640, are all about three times as bright as in the Sun; hence, the basic structure of the outer atmosphere of this prototype K dwarf bears similarities to that in the Sun. The factor of 3 increase in brightness of the chromospheric lines has been explained by a steeper temperature gradient in the lower

chromosphere¹⁵, which results in higher densities and thus more collisional excitation of the lines. If the transition region in ϵ Eri is geometrically thin and conductively heated as in the Sun, then the strength of its lines is proportional to the transition region pressure⁷ and this pressure should be a factor of 3 larger in ϵ Eri than in the quiet Sun. λ And is similar to ϵ Eri except that there seems to be twice as much material in the transition region.

Table 4 Line surface fluxes/average quiet Sun

Ions	Temperature	ϵ Eri	λ And	α Aur	HR1099	Ref.
C I, Si I, Si II	$4-6.5 \times 10^3$	2.4	4.2	4.9	70	This work
O I $\lambda\lambda 1302, 4, 6$	$\sim 6 \times 10^3$	3.8	16	19	63	This work
Ca II, Mg II	$\sim 6 \times 10^3$	2.0	4.6	3.7	50	2, 15, 22
Lya	2×10^4	1.3*	3.9*	1.5*	50*	5, 22, 26
C II, C III	$4.5-9 \times 10^4$	3.3	4.7	20	100	This work
He II	$\sim 8 \times 10^4$	12	22	20	190	This work
C IV, Si IV, N V	$0.8-3 \times 10^5$	4.4	9.5	26	84	This work

* Uncorrected for interstellar absorption.

Capella and HR1099, by comparison, are very different from the quiet Sun. Capella shows considerably more material in its transition region than the Sun, whereas HR1099 shows large enhancements in both the transition region and the chromosphere. Since the surface fluxes for HR1099 are enhanced over the quiet Sun fluxes by a factor of 10^2 , similar to the typical enhancement of permitted lines during solar flares³⁴, a solar-like flare may have covered most of the stellar surface or a far more intense flare may have covered a small portion of the surface during this episode.

Third, for Capella the line surface fluxes for ions formed at $T > 3 \times 10^4$ K are typically 20 times larger than for the quiet Sun. By comparison, Vitz *et al.*⁵ obtained values only 10 times larger than for the quiet Sun. This apparent factor of two discrepancy is due mainly to the different angular diameters for Capella A assumed here ($0.0072''$) and by Vitz *et al.*⁵ ($0.0094''$) in converting observed flux to surface flux. Measured fluxes at Earth are in good agreement (within 15%) for the $\lambda\lambda 1206, 1240, 1305$, and 1550 features, but the IUE values are about 65% larger for the $\lambda\lambda 1335$ and 1397 features formed by the C II and the Si IV and O IV lines. We cannot say with certainty at this stage whether the discrepancy in the $\lambda\lambda 1335$ and 1397 fluxes is due to measurement error or represents real changes in the emission from Capella. We suspect, however, that the discrepancy results from the preliminary nature of our calibration scheme, as the $\lambda 1550$ fluxes, due mainly to C IV, agree and there is no evidence for large changes in emission-line fluxes with phase except for O VI.

Fourth, in the high-resolution Capella spectrum there is no systematic trend in wavelength residual with ionisation potential or presumed temperature of maximum abundance. Thus we see no evidence for the onset of a stellar wind at temperatures of $100,000-200,000$ K, as previously seen in the O VI $\lambda 1032$ line (formed at $300,000$ K) by Dupree⁴ at phase 38.2 d.

Fig. 5 Reduced profiles of the Capella C II $\lambda\lambda 1334, 1335$ lines in absolute flux units

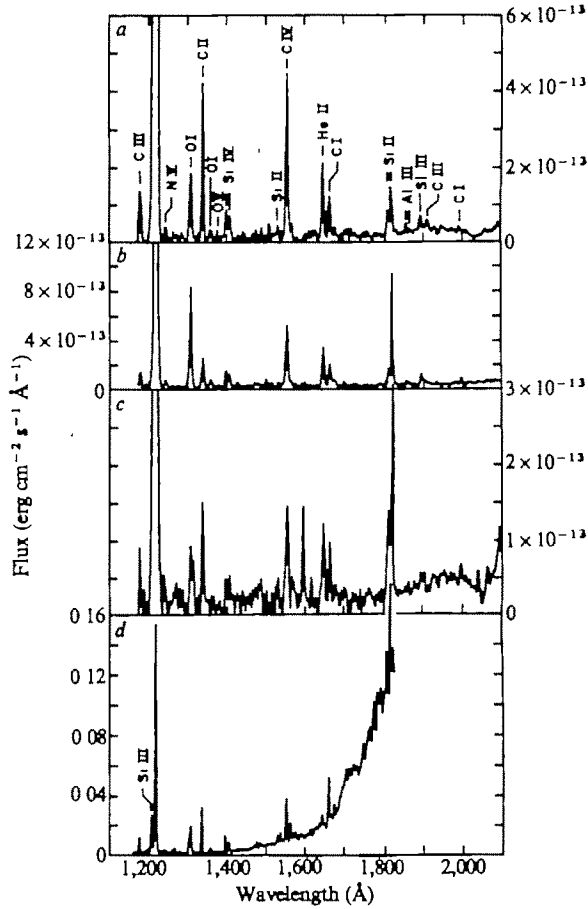
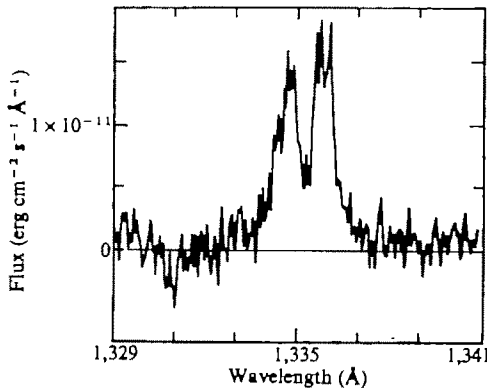


Fig. 6 Low-dispersion short-wavelength spectra of a, HR1099, b, λ And, and c, ϵ Eri in absolute flux units are compared with the quiet Sun (d) spectrum degraded to $6 \text{ \AA}$ resolution. Important lines are identified. The stellar Ly α and Si III $\lambda 1206$ lines are covered by saturated geocoronal Ly α emission.

There is, however, a significant increase in line width (FWHM) with ionisation potential—C I ($0.28 \text{ \AA}$), O I ($0.47 \text{ \AA}$), Si II ($0.43 \text{ \AA}$), C II ($0.82 \text{ \AA}$), Si III ($0.69 \text{ \AA}$), C III ($0.54 \text{ \AA}$), Si IV ($0.67 \text{ \AA}$), C IV ($0.89 \text{ \AA}$), and N V ($0.71 \text{ \AA}$). These widths, except for C I and N V, are probably accurate to $\pm 10\%$. This trend suggests that turbulent motions increase with height in the outer atmosphere as occurs in the Sun¹⁵. The C II and C IV lines appear to be anomalously broad compared to the trend, perhaps due to significant optical depths in these lines.

In Capella, the emission lines are all single, as would be expected at phase 77.711 d (based on the orbital elements of Batten and Erceg)³⁵ when the radial velocity difference between the two stars is very small. Also, no lines seem to show a narrow component (from Capella A) superimposed on a broader component (from the more rapidly rotating Capella B)^{36,37}. Thus we cannot ascribe the observed spectrum to a specific star on the basis of our data alone, but instead rely on previous work^{3,4} for evidence that Capella A is the emitting star.

Finally, line flux ratios for O I $\lambda 1306/\lambda 1304/\lambda 1302$ and C II $\lambda 1335/\lambda 1334$ in Capella are close to unity and similar to the solar ratios, implying large optical depths in the lines. On the other hand, the C IV $\lambda 1550/\lambda 1548$ ratio is 2.1, similar to the solar ratio, 1.8, and to the ratio of *gf* values for the lines, 2.0. We conclude that these lines must be effectively thin (not thermalised), but that the anomalous width of the lines may be due to optical-depth effects. The Si II $\lambda 1817/\lambda 1808$ ratio is 3.1 in Capella, 2.8 in ϵ Eri, and 2.4 in HR1099, compared to the *gf* value ratio, 2.0. The fact that these ratios are in excess of the optically thin value is at first sight surprising, but may be explained if the lines at $1,808.4$ and $1,816.9$ become optically thick whilst the $1,817.4$ component remains optically thin. Photons may then be transferred from $1,804.4$ to $1,817.4$, thus increasing the ratio of $1,817.4$ plus $1,816.9$ to $1,808.4 \text{ \AA}$.

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IUE observations of the interstellar medium

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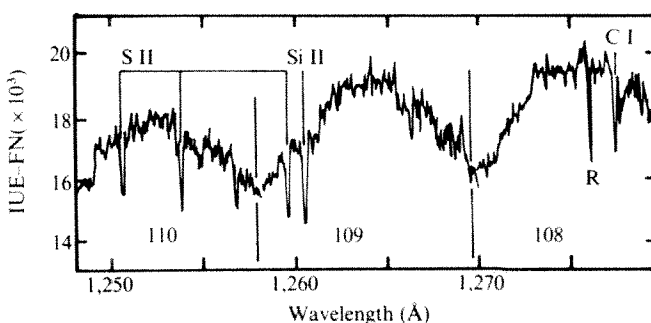
Results from a preliminary study of interstellar absorption lines in the spectra of the three stars HD149757, HD93521 and HD153919, observed by the IUE during its commissioning phase are presented. They demonstrate the capabilities of the IUE for interstellar research. Results from a preliminary study of the emission line spectrum of the planetary nebula NGC7027 are outlined and used to study its interstellar reddening.

THE IUE with its wide spectral coverage, roughly 1,150–3,200 Å embracing many of the resonance lines of the common elements, its excellent sensitivity throughout this band, and its large resolving power of 10^4 when used in the high dispersion mode, is a unique instrument for studying the interstellar medium. Atomic species that can be observed include H I, D I, Be I–II, B I–III, C I, C II, C IV, N I, N V, O I, Na I, Mg I–II, Al I–III, Si I–IV, P I–III, S I–III, Cl I, Ca I–II, Sc III, Ti I–III, V I–III, Cr I–II, Mn I–II, Fe I–II, Co I–II, Ni I–II, Cu I–II and Zn I–II. In some cases, such as C I, C II, O I, Si I, Si II, Fe II, not only the resonance lines, but also lines originating from excited fine-structure levels within the ground state are accessible. In addition to the atomic species, several diatomic and polyatomic molecules which are thought to be abundant in interstellar clouds have lines within the IUE band. Obvious candidates for such observations are OH, CO, C₂ and CH; the first, with its

transition near 3,080 Å, and the last, with its transitions near 3,137–3,146 Å, are also accessible from the ground. Some of the rarer molecular species which still might be detected in favourable circumstances include H₂O, MgH⁺, CH₃, CO⁺, NO, NO⁺, SiO and CS.

In the low dispersion mode IUE can also provide important new information about the absorption and scattering characteristics of interstellar dust.

Fig. 1 Observed spectrum of HD149757 without background and echelle ripple correction. The section of the spectrum contained in orders number 110–118 of the short-wavelength spectrograph is plotted showing strong narrow absorption lines due to interstellar C I, Si II and S II. The features indicated by R are due to fiducial marks. Vertical lines mark the ends of echelle orders.



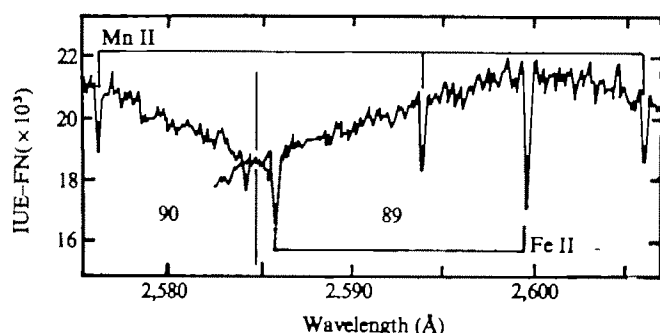


Fig. 2 Spectrum of HD149757 contained in orders number 90-89 of the long-wavelength spectrograph, showing strong absorption lines due to interstellar Mn II and Fe II.

During the commissioning phase of IUE the spectra of three stars, HD93521, HD149757 and HD153919, were obtained in order to study their interstellar absorption lines. As a fourth target the planetary nebula NGC7027 was observed in order to study its emission line spectrum. Table 1 lists these targets and the observations obtained.

The star HD149757 (ζ Oph) has been selected as a prime target for the study of interstellar absorption lines for many reasons. It is a bright source, it has been exceedingly well studied in the UV, in particular by the Copernicus satellite¹, thus providing an excellent basis to demonstrate the IUE capabilities in comparison with these other instruments. It also has a large H_2 column density, suggesting that other molecules might be found along this line of sight. As the Copernicus observations did not cover all UV wavelengths equally well, the IUE observations will extend the earlier data.

HD93521 was observed as a photometric standard as well as for the study of interstellar absorption lines. It is one of the few

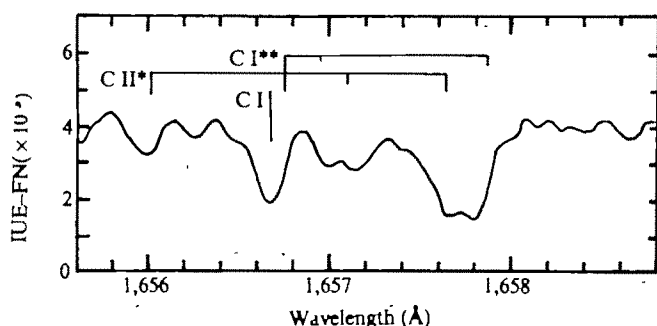


Fig. 3 The spectrum of HD149757 in the neighbourhood of the C I λ 1656.928 line, corrected for the inter-order background. Note the strength of the absorption lines originating from excited levels within the ground state of C I (* and ** indicate 1st and 2nd excited levels). The wavelength scale plotted is the one assigned during the data reduction process. The laboratory wavelengths indicated on the figure have been shifted by a constant amount of -0.25 Å to correct for an offset in this scale.

early type stars known to be at a large distance from the galactic plane at $z = 1,570 \text{ pc}$. Also, its interstellar line spectrum shows a multiple component structure with a velocity separation of several tens of km s^{-1} (ref. 2), which is resolvable by the IUE.

HD153919 was primarily observed as it is the optical counterpart of an X-ray source (3U1700-37); and is discussed by Dupree *et al.*³ in the following article. However, it is one of the most highly reddened stars observed by IUE during the commissioning phase making it an excellent candidate for a search for interstellar absorption lines from rare species.

Absorption lines in the spectra

We now present illustrative results from a study of interstellar absorption lines in the spectra of the three stars HD149757, HD93521 and HD153919. The analysis is preliminary and based on data that had not been fully processed at the time.

HD149757

Figures 1 and 2 show two 30 Å bands at $1,250\text{--}1,280 \text{ Å}$ and $2,575\text{--}2,605 \text{ Å}$ extracted from the short-wavelength and long-wavelength spectrograms. The first of these covers the echelle orders 110-108 in the short-wavelength spectrograph and the data are uncorrected for the echelle ripple. The second covers the orders 90-89 in the long-wavelength spectrograph. In both cases, the strong interstellar lines from S II, Si II, Mn II and Fe II are clearly visible and have an equivalent width of the order of 100 mÅ . In Fig. 3 is plotted a 3 Å segment of the short-wavelength spectrograph around $1,657 \text{ Å}$ where several C I lines occur from either the ground level proper or from excited levels within the ground state term. The equivalent width of the C I λ 1656.928 line is $\sim 70 \text{ mÅ}$. Note that the wavelength scale plotted is the one assigned during the data reduction process. It has an offset of $\Delta\lambda = 0.25 \text{ Å}$ which has been taken into account when locating the expected line positions.

In Fig. 4 the data for five spectral lines observed by the Copernicus satellite are compared with those observed by the IUE. These lines and their equivalent widths as measured by Morton¹ are S III λ 1190.206 (67 mÅ), Si II λ 1190.418 (132 mÅ), P II λ 1301.87 (15.6 mÅ), O I λ 1302.169 (201 mÅ) and Mg II λ 2802.704 (290 mÅ). The Copernicus profiles have been obtained by averaging two, three and two scans, respectively, whereas the IUE data are from a single image. Figure 4 demonstrates that the resolution of the IUE as compared to the Princeton spectrophotometer is somewhat degraded but that features separated by 0.2 Å can still be clearly resolved. Also note the much lower noise in the Mg II λ 2802 line, as observed by the IUE.

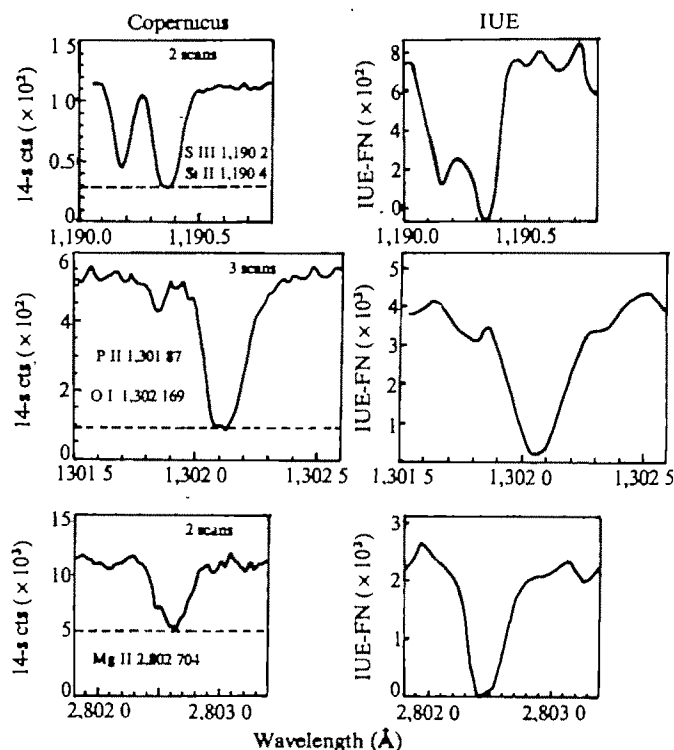


Fig. 4 Comparison of spectral line profiles as observed by the Copernicus satellite (Morton 1974) and by IUE in the spectrum of HD149757. The IUE wavelength scale has not been corrected for an offset.

Table 2 shows a comparison of the equivalent widths measured by the two systems. A statistical assessment shows that the IUE values are on average 10% higher than Copernicus and the r.m.s. deviation of the ratio of the two is $\pm 16\%$. This agreement is very satisfactory, particularly as the IUE data reduction and calibration were in their early stages and most of the large differences do occur for lines which are at present difficult to measure on IUE spectra.

Table 1 Targets for interstellar line observations

Object	(V)	(Sp T)	(E(B-V))	Image 1				IUE observations Image 2				Image 3			
				1	2	3	4	1	2	3	4	1	2	3	4
HD93521	6.89	0.9 V	0.03	SWR	HR	SA	30								
HD149757	2.56	0.9 V	0.32	SWR	HR	SA	22	LWP	HR	SA	2	LWP	HR	SA	7
HD153919	6.55	0.6 f	0.58	SWR	HR	LA	100	LWP	HR	LA	30-				
NGC7027	10	—	—	SWR	LR	LA	60	LWR	LR	LA	120	LWR	HR	LA	180

* 1, Camera, 2, Spectrograph, 3, Aperture, 4, Exposure time (min).
LWP, long-wavelength prime; LWR, long-wavelength redundant; SWP, short-wavelength prime, SWR, short-wavelength redundant
HR, high resolution; LR, low resolution SA, small aperture; LA, large aperture.

HD93521

To study interstellar matter well above the galactic plane, the halo star HD93521 has been observed. It is located at $b^{\text{II}} = 62^\circ$ at a distance of 1,778 pc, giving $z = 1,570$ pc. The absorption lines originating from the local gas and the high z gas have a large velocity separation. The strongest components occur at v (heliocentric) = -12 km s^{-1} and -56 km s^{-1} with additional components at $+5 \text{ km s}^{-1}$ and -34 km s^{-1} (see ref. 2). For the strongest two components the HI column density is $2 \times 10^{19} \text{ cm}^{-2}$ and $8 \times 10^{19} \text{ cm}^{-2}$ (ref. 4). H_2 has been detected in the -12 km s^{-1} component⁵.

Figures 5 and 6 show the absorption line profiles as observed by IUE. For comparison the Ca II K-line profile measured by Münch and Zirin² is included in Fig. 5. In plotting the IUE results we have kept the wavelength scale as assigned during the data reduction process but shifted the marks that indicate the expected line position by -56 km s^{-1} and -12 km s^{-1} with respect to this scale. The main components are clearly seen in the C I $\lambda 1277.245$, C II $\lambda 1334.532$, C II $\lambda 1335.708$ and Si II $\lambda 1259.520$ lines. In these cases the -56 km s^{-1} component seems to be weaker than the -12 km s^{-1} one, consistent with the optical and HI results. In the case of the Si II $\lambda 1190.418$ and S III $\lambda 1190.206$ lines the same may hold but a deconvolution of

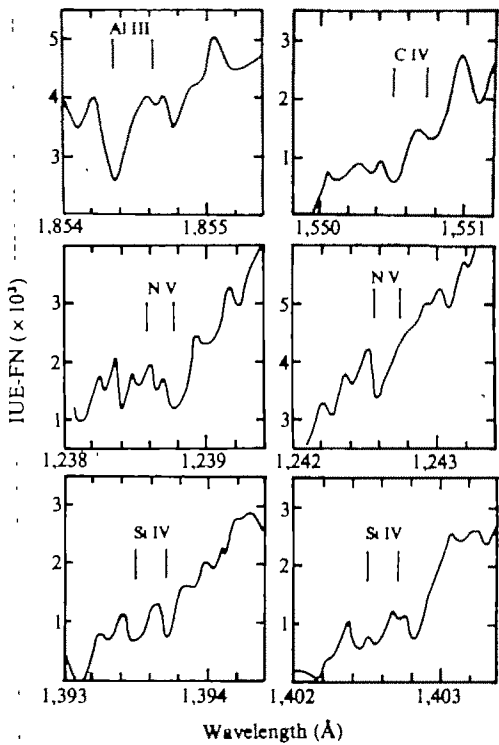
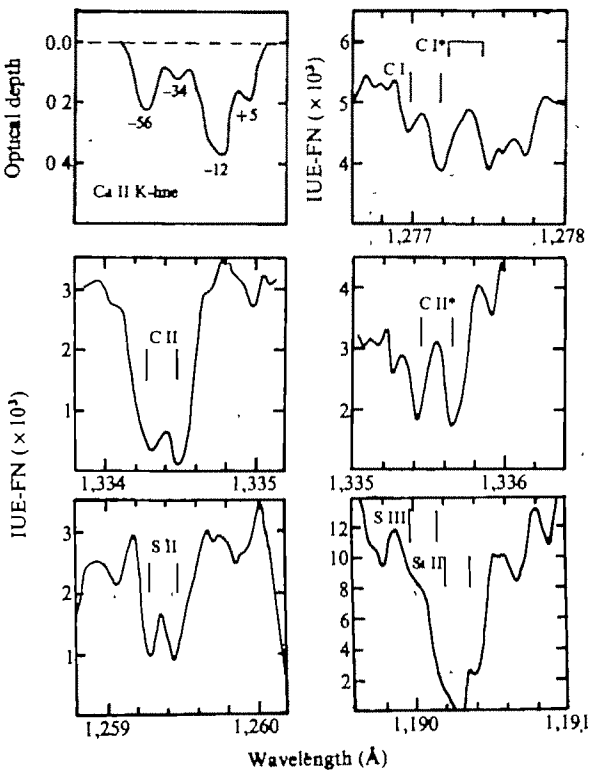


Fig. 6 As Fig. 5, except that interstellar absorption lines originating from highly ionised species have been plotted



the observed profiles would be required to demonstrate this. An enhanced strength of the -56 km s^{-1} component which is associated with the gas at $z \approx 500$ pc (refs 2, 4 and 6) is apparent in the Al III $\lambda 1854.720$ line. The same may be true for the C IV $\lambda 1550.774$ and Si IV $\lambda\lambda 1393.755, 1402.769$ lines. However, the N V $\lambda\lambda 1238.821, 1242.804$ lines do not seem to follow this trend. Indeed there is no strong evidence for the presence of interstellar N V.

HD153919

HD153919 was observed primarily as an X-ray source and a more extensive discussion of its interstellar characteristics is given by Dupree *et al.*³. It shows the richest interstellar absorption line spectrum of the three stars observed. Not only are the lines considerably stronger than those in the spectrum of HD149757, which is expected from the higher reddening, but the lines also indicate a wider range of ionisation along this line of sight. Most noticeable is the strength of the lines arising from such highly ionised species as C IV and Si IV.

Figures 7 and 8 show two $30 \text{ \AA}$ sections of the spectrum of HD153919. Strong narrow absorption lines from singly ionised species like Si II, P II, Mn II and Fe II are clearly visible as well as the C IV lines at $1,550 \text{ \AA}$. As indicated in Fig. 7, lines arising from excited levels within the ground state are strong in this spectrum. This is even more evident from Fig. 9, where we have plotted the spectrum in the neighbourhood of the C I $\lambda 1656.928$ line. Figure 10 shows the evidence for the more highly ionised

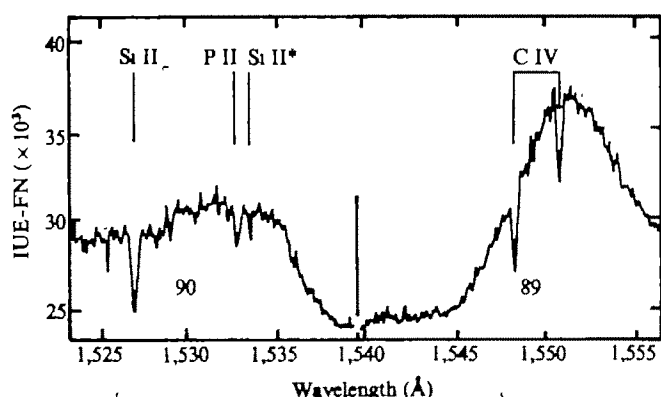


Fig. 7 Observed spectrum of HD153919 with neither background nor echelle ripple correction. The section plotted is contained in the echelle orders number 90-89 in the short-wavelength spectrograph and shows evidence for strong interstellar absorption lines due to single as well as multiple ionised species

species such as Si III, Al III, C IV and Si IV. The presence of N V remains doubtful. X-ray ionisation of the interstellar medium local to this source might explain the presence of these high ion stages^{3,7}.

Emission lines in the spectrum of NGC7027

NGC7027 has been selected as a prime target for the study of its emission lines in the UV on several grounds. First, it was known to exhibit a strong UV line spectrum from the work of Bohlin *et al.*⁸. These data have been obtained with a rocket-borne 33 cm telescope feeding a spectrograph that yielded 46 Å mm⁻¹ dispersion in the 1,300-2,900 Å region. Second, with its angular size of 10 arc s, the nebular image fits nicely in the 10×20 arc s entrance slot of the IUE spectrograph. Third, and perhaps most

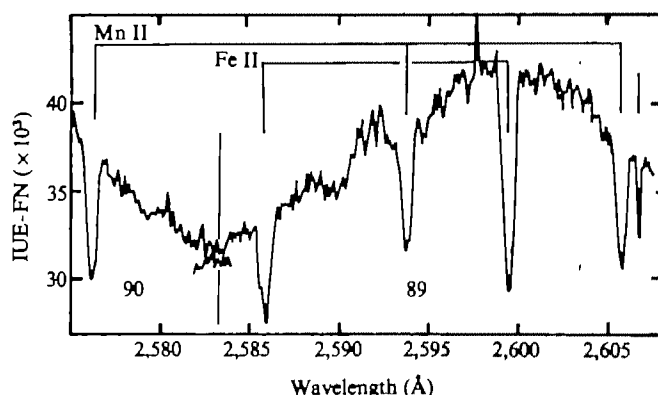


Fig. 8 As Fig. 7, except that a 30 Å section from the long-wavelength spectrograph of HD153919 has been plotted, showing the great strength of the Mn II and Fe II lines (see Fig. 2).

important, NGC7027 is now the one planetary nebula for which the emission spectrum has been studied all the way from the radio band⁹, through the near IR (10-12.5 μm (ref. 10); 4-8 μm (ref. 11); 2-4 μm (ref. 12); 0.8-2.7 μm (ref. 13)), the optical (Kaler *et al.*¹⁴ discuss the entire band from 3,050 to 8,600 Å), into the UV. These extensive data should provide an excellent basis for the detailed comparison with model calculations for this planetary nebula.

NGC7027 was observed three times with IUE. Low resolution spectra of the long-wavelength and the short-wavelength range and a high resolution spectrum of the long-wavelength range were obtained. Figure 11 shows the low resolution spectra. The intensities have not been corrected for the IUE sensitivity response but the zero point of the wavelength scale was corrected on the basis of identified lines.

The most notable feature in the spectrum is the great strength of the emission lines, most of which are readily identified. These are listed in Table 3, together with preliminary flux values. The fluxes were determined from sensitivity curves derived from observations of standard stars as described by Boggess *et al.*²³ but with the TD-1 (S2/68) fluxes as standard²⁴. The relative line intensities within each spectrum should be accurate to better than ±30% for the stronger features, and roughly ±50% for the weaker ones, except for λ > 3,100 Å where the sensitivity is low and the ±50% error applies to all lines. To combine the data from the two spectral ranges, the long-wavelength data were fitted to the short-wavelength data by using the intensity of the

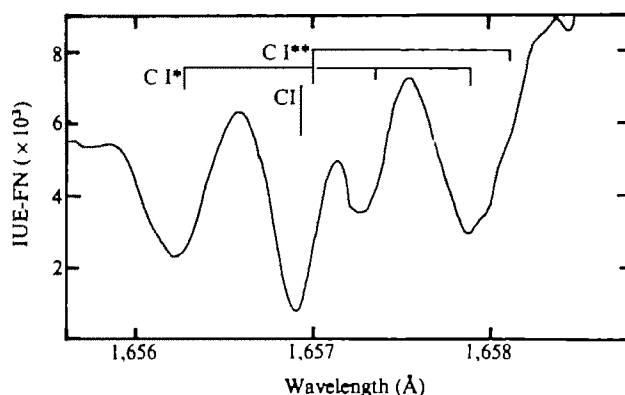


Fig. 9 The spectrum of HD153919 in the neighbourhood of the C I λ1656.928 line. Note the great strength of the absorption lines originating from excited fine structure levels within the ground state of C I. The wavelength scale is the one assigned during the data reduction process

C III λ1909 line which is seen in both spectra. A comparison of these results with those from other experiments shows excellent agreement with the intensities as observed by the ANS satellite¹⁵ for the C IV λ1550 line, and consistent agreement with the results from the rocket experiment of Bohlin *et al.*⁸, which are, however, systematically smaller, by a factor of ~2. For λ >

Table 2 Equivalent widths for strong lines in the spectrum of HD149757 as measured by Copernicus (COP) and IUE

Ion	λ _{Lab} (Å)	W _λ (COP) (mÅ)	W _λ (IUE) (mÅ)	Ion	λ _{Lab} (Å)	W _λ (COP) (mÅ)	W _λ (IUE) (mÅ)
C II	1,334.532	189	224	Si II	1,193.289	147	75†
C II*	1,335.703	140	184†	Si II	1,190.416	132	130
N I	1,200.711	133	194†	S II	1,259.520	112	148†
N I	1,200.224	131	151	S II	1,253.812	106	112
N I	1,199.549	146	167	S II	1,250.586	100	105
O I	1,302.169	201	198	Mn II	2,605.697	111	115
Mg I	2,852.127	218	196	Mn II	2,593.731	116	163
Mg II	2,802.704	290	337†	Mn II	2,576.107	138	137
Mg II	2,795.528	312	309	Fe II	2,599.395	226	247
Si II	1,526.708	190	156	Fe II	2,585.876	214	174
Si II	1,304.372	132	127	Fe II	2,382.034	238	259
Si II	1,260.421	170	223‡	Fe II	2,373.733	120	185†

As strong lines we have arbitrarily defined lines that have an equivalent width of more than 100 mÅ as measured by Morton in the Copernicus spectrum of HD149757. Uncertainties (denoted by †) arise in the IUE data if the line is close to the end of an order or in a badly exposed part of the image. The Si II λ 1,260.421 line is blended by the Fe II λ 1,260.542 line (denoted by ‡).

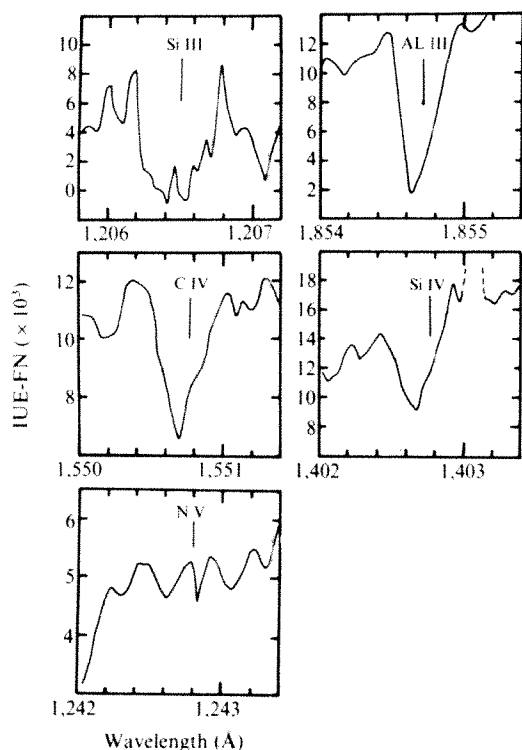


Fig. 10 Sections from the short-wavelength spectrogram of HD153919 showing evidence for interstellar absorption lines originating from highly ionised species. The raw wavelength scale is plotted.

3,100 Å, where the calibration is still rather uncertain, the ANS results and the measurements of Kaler *et al.*¹⁴ suggest that the IUE fluxes are systematically too low by 25–45%.

Figure 12a plots, on an enlarged scale, the 2,760–2,820 Å section of the low resolution spectrum showing a broad extended hump which contains the Mg II λ 2795, 2803 doublet. Figure 12b shows the same portion of the spectrum as observed by the high resolution mode. The wavelength scale is, in both cases, the uncorrected scale assigned during the reduction process. The two doublet lines as well as the [Mg V] line at λ 2783 Å are clearly seen. This example shows the potential of the high resolution mode of IUE for studying the emission from planetary nebulae in great detail.

While the low resolution short-wavelength spectrum of NGC7027 resembles in many ways the spectrum of NGC7662 as observed by Bohlin *et al.*¹⁶ in that C IV λ 1550, He II λ 1640 and C III λ 1909 are the most prominent features, the low resolution long-wavelength spectrum looks by comparison much richer in emission lines. Bohlin *et al.* quote the

Table 3 UV line fluxes in the spectrum of NGC7027

(Å)	Ion	Obs. flux (10^{-12} erg cm $^{-2}$ s $^{-1}$)	(Å)	Ion	Obs. flux (10^{-12} erg cm $^{-2}$ s $^{-1}$)
1,406	O IV]	0.8*	2,783	[Mg V]	1.0†
1,487	N IV]	0.5*	2,795	Mg II	1.6†
1,549	C IV	20.4*‡	2,803	Mg II	1.6†
1,602	[Ne IV]	1.1*	2,836	O III	0.7†
	+				
1,640	He II	5.2*	2,853	[Ar IV]	0.2†
1,663	O III]	0.9*	2,868	[Ar IV]	0.1†
1,750	N III]	0.8*	2,928	[Mg V]	0.2†
1,909	C III]	10.8*‡	3,024	O III	0.4†
2,321	[O III]	0.9†	3,047	O III	1.5†
2,422	[Ne IV]	1.3†	3,133	O III	8.3†‡
2,470	[O II]	0.4†	3,203	He II	2.2†
2,511	He II	0.2†	3,345	[Ne V]	5.5†
2,734	He II	0.9†	3,426	[Ne V]	†

* Seen in the short-wavelength spectrum

† Seen in the long-wavelength spectrum

‡ Some pixels are saturated

Table 4 UV continuum fluxes in NGC7027

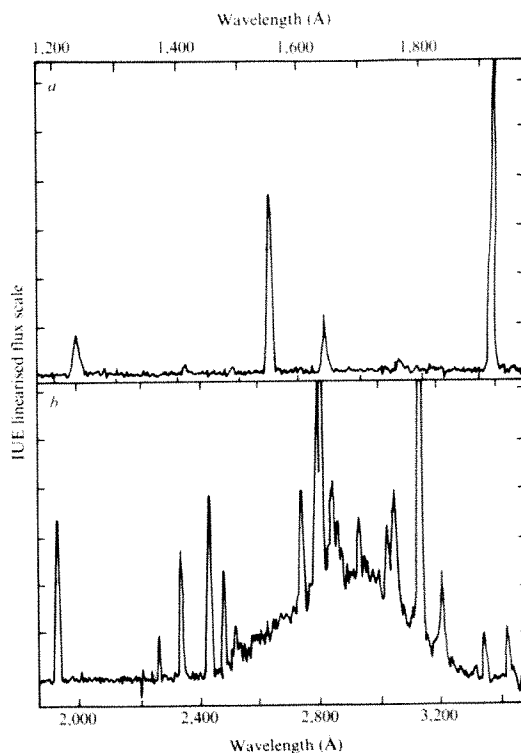
(Å)	Observed flux (10^{-15} erg cm $^{-2}$ s $^{-1}$ Å $^{-1}$)	(Å)	Observed flux (10^{-15} erg cm $^{-2}$ s $^{-1}$ Å $^{-1}$)
1,400	13	2,400	4
1,600	15	2,600	10
1,800	12	2,800	20
2,000	14	3,000	36
2,200	6	3,200	46

wavelengths of the components of the [Ne IV] $^2D_{3/2} \rightarrow ^4S, ^2D_{5/2} \rightarrow ^4S$ doublet to be 2,439 and 2,441 Å. However, Edlén (ref. 17 and personal communication) gives these wavelengths as 2,422.60 and 2,425.24 Å, from isoelectronic sequence extrapolations. The high resolution spectrum shows the doublet to be clearly resolved, and the wavelengths to be in agreement with Edlén's prediction, to within the accuracy of the present measurements (~ 0.5 Å). The intensity ratio $R = I(\lambda 2425)/I(\lambda 2423)$ is sensitive to electron density, being similar to the well known [O II] ratio $I(\lambda 3729)/I(\lambda 3726)$. In the limit of low densities, $R = 1.5$, and in the limit of high densities, $R = 0.16$ (the latter value being obtained using the transition probabilities of Garstang¹⁸). For NGC7027 we estimate R to be about unity (see Fig. 13) which gives a value of electron density of about 5×10^4 cm $^{-3}$.

Table 4 lists preliminary estimates of the UV continuum fluxes from NGC7027. Although the modification of the intensity distribution by extinction is substantial, the data resemble the Edistribution expected from hydrogen recombination and two-photon emission.

The UV emission lines of NGC7027 can also be used in a study of interstellar absorption. This is usually investigated by comparing reddened and unreddened stars which are believed to be intrinsically identical from their line spectra. Planetary nebulae provide a totally different method in that the recombination lines in H I and He II can be linked directly to the free-free radio emission. Because the interstellar medium is optically thin to the latter, its measured flux gives the intrinsic

Fig. 11 The low resolution *a*, short- and *b*, long-wavelength spectra of NGC7027 as observed by IUE. The flux values have not been corrected for the instrumental sensitivity. For an identification of the emission lines and corrected flux values see Tables 3 and 4.



fluxes in the H I and He II lines. A comparison with the observed line fluxes then gives directly the interstellar extinction.

NGC7027 is particularly appropriate to such a study because it is heavily reddened and has been observed extensively. Previous studies by Miller and Matthews¹⁹ used radio data and the H α , H β and H γ lines, and by Schwartz and Peimbert²⁰ who included lines at $\lambda = 1 \mu\text{m}$. With the observation of the UV He II lines with IUE an investigation of interstellar extinction over a very wide wavelength range is now possible and a brief analysis is given here. A more extensive and detailed discussion will be given elsewhere²¹.

The values of the interstellar extinction in NGC7027— $A_\lambda(7027)$ —have been derived from the IUE observations of He II lines on the basis of the method outlined above and combined with those obtained from ground-based data. The results are plotted in Fig. 14 against the values of A_λ/E_{B-V} derived by Nandy *et al.*²² from a study of early-type stars with the sky survey telescope (S2/68) on the TD-1 satellite. The excellent straight line fit of the data shows that over a wide range of wavelengths, from H α 6,563 Å to He II 1,640 Å, the reddening of NGC7027 follows closely the standard reddening law as derived by Nandy *et al.*²².

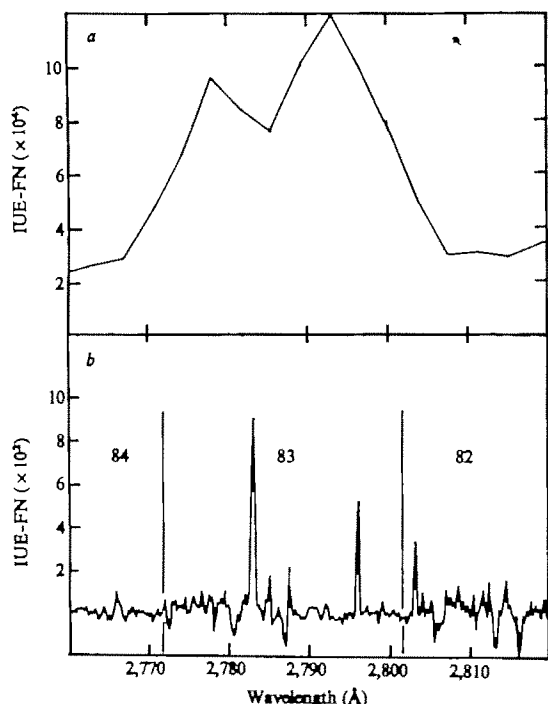


Fig. 12 Comparison of *a*, low resolution and *b*, high resolution spectra of NGC7027 in the neighbourhood of the Mg II $\lambda 2800$ doublet. The high resolution spectrum is extracted from the echelle orders number 84–82. The wavelength scale is the one assigned during the data reduction process.

For the longer wavelengths there is some discrepancy between the extinction deduced for NGC7027 and the standard extinction. The straight line of Fig. 14 does not pass through the origin and can be represented by

$$A_\lambda(7027) = \alpha \left(\frac{A_\lambda}{E_{B-V}} \right) + \beta$$

where $\alpha = 0.935$ and $\beta = 0.17$. The difference is small but there is no doubt that it is real.

An explanation of this discrepancy can be made by considering the large variation of extinction across the face of the nebula which is known to exist from the quite different distributions in the radio and optical images. Let us consider a simple two-component model in which there is a heavy local extinction over a fraction B of the total area of the source. We take the

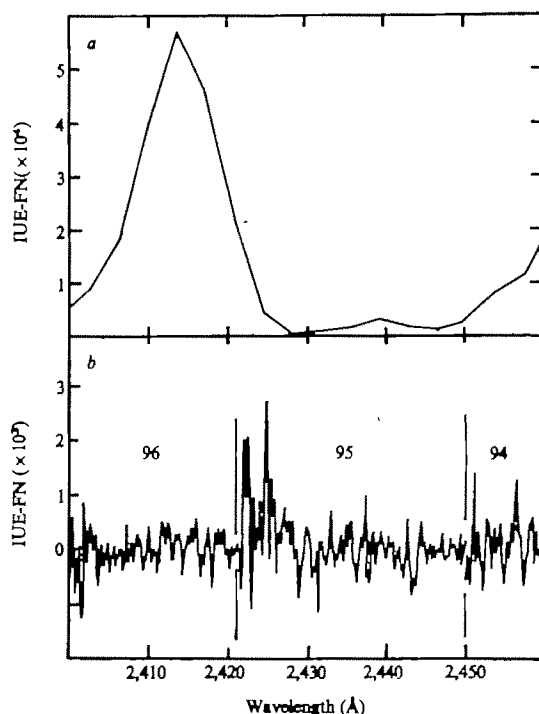


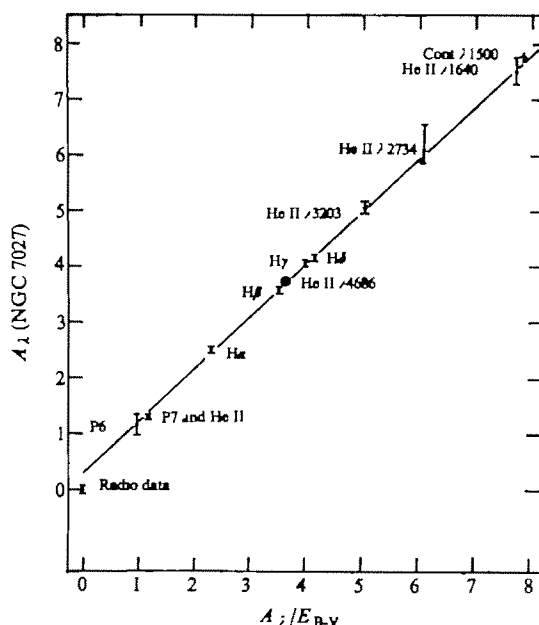
Fig. 13 As Fig. 12, except that the spectrum in the neighbourhood of the [Ne IV] doublet has been plotted.

extinction from area B to be $\gamma(A_\lambda/E_{B-V})$ and from the remaining $(1-B)$ to be $\alpha(A_\lambda/E_{B-V})$ with $\alpha = 0.94$ as derived above and $\gamma \gg \alpha$. We can then write

$$10^{-0.4 A_\lambda(7027)} = B 10^{-0.4 \gamma (A_\lambda/E_{B-V})} + (1-B) 10^{-0.4 \alpha (A_\lambda/E_{B-V})}$$

Assuming the first term to be negligible over the wavelength range 6,563–1,640 Å where the straight line fit is good, then the two-model equation is consistent with that fit if $(1-B) = 10^{-0.4 \beta}$, giving $B = 0.145$. The value of γ can now be deduced by fitting in the red and IR regions. Using the data plotted in Fig. 14 together with the most extreme IR measurement yet available (Brackett γ at 2.166 μm , Merrill *et al.*¹²), a good fit is obtained with $\gamma = 2.0$. Hence, this simple, two component model gives

Fig. 14 Interstellar extinction measured for NGC7027 in the radio, IR, optical and UV ranges. A_λ for NGC7027 has been plotted against (A_λ/E_{B-V}) as determined by Nandy *et al.*²² from observations of about 100 reddened early type stars with the Sky Survey Telescope (S2/68) in the ESRO TD 1 satellite.



excellent agreement with the observations over the full wavelength range from the IR to the UV.

The reddening derived for NGC7027 over the range 6,563–1,640 Å is in excellent agreement with that derived from early type stars by Nandy *et al.* Most of the reddening is probably due to interstellar grains along the line of sight. The results for larger wavelengths can be explained by a local extinction, variable over the nebula, caused by grains with the same optical properties.

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IUE observations of X-ray sources: HD153919 (4U1700–37), HDE226868 (Cyg X-1), HZ Her (Her X-1)

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The first UV spectroscopic measurements of binary X-ray sources with IUE show highly variable emission from a photoionised plasma in the object HZ Her, and give evidence for localised circumsystem material in the binary source Cygnus X-1. In addition, a substantial stellar wind is found in one of the brightest identified X-ray sources HD153919. This system may be surrounded by an extensive H II region unusual in its content of high excitation ion species.

BINARY X-ray sources containing a compact object represent a class of stars that generally have been inaccessible to UV spectroscopic observations due to their faintness. Studies of these objects can potentially discern the physical conditions and dynamics of the material in the sources themselves as well as probe the interaction between the source and the surrounding interstellar medium. The IUE was used during the high priority phase of the commissioning period to obtain spectra of three stars—HD153919, HDE226868, HZ Her—that correspond to the X-ray sources 4U1700–37, Cyg X-1, and Her X-1 respectively. We report here some general characteristics of the spectra, comments on the presence of phase effects, and note the

strength of interstellar lines towards one source, HD153919, where high resolution spectra are available

Observations

A discussion of the instrument and its performance is contained in preceding articles^{1,2}. The spectra discussed here were taken over a 7-week period beginning 12 February 1978 (see Table 1). All exposures were made with the large aperture. Corrections for the effects of the radiation background and scattered light were applied by subtracting a smoothed value of the inter-order fluxes from the spectrum in each order. The adequacy of this correction for the high dispersion spectra is verified by the appearance of strong interstellar lines (that is, H Ly α at λ 1216 and C II at λ 1335) and absorption components of P Cygni profiles with flat cores of approximately zero net flux. The high dispersion spectra of HD153919 have not been corrected for the grating blaze (echelle ripple); the low dispersion spectra have been calibrated by using the preliminary determination of the instrumental sensitivity with wavelength³.

HD153919 = 4U1700–37

This binary system identified with the X-ray source⁷ is composed of a primary star classified from the optical spectrum as O6f, and a compact object—the X-ray source—orbiting essentially within

the expanding atmosphere of the primary star⁸. This source was observed at high dispersion at two phases ~ 0.35 and 0.97 , in its 3.4-d binary period.

The spectrum of the primary star shows the characteristic rich array of P Cygni profiles that has been found in other Of stars (see refs 9, 10). The lines of the strong resonance doublets of C IV ($\lambda 1550$) and Si IV ($\lambda 1400$) are shown in Figs 1 and 2 where the broad extent of these lines is apparent. Transitions that show P Cygni features and some characteristics of their profiles at phase 0.97 are given in Table 2. Here the velocity scale has been reduced to the centre-of-mass velocity of the system (-60 km s^{-1}) by using interstellar lines of low excitation and assigning them the measured heliocentric velocity of the Ca II interstellar lines¹¹, namely -14.7 km s^{-1} . The positions of line maxima were measured from tracings on a scale of $4 \text{ \AA}$ per inch; measurements to $\pm 0.1 \text{ \AA}$ can be achieved. A greater uncertainty arises in selecting the appropriate positions on the profiles since effects of the grating blaze have not been removed from these scans. This can cause a shift in the apparent maximum of a P Cygni profile and errors in the assignment of the shortwards edge. An uncertainty of $\pm 100 \text{ km s}^{-1}$ is probably a reasonable estimate of the errors in this procedure. The observed terminal velocities $\sim 2,600 \text{ km s}^{-1}$ are comparable to those found in ζ Pup (ref. 12) (spectral type O4 If) and are larger than velocities occurring in other O supergiants of later spectral type¹⁰, for example, 29 C Ma (O7 Ia:f), 9 Sge (O7.5 Ia:f), and HD151804 (O8 Ia:f).

The escape velocity from HD153919 is $\sim 720 \text{ km s}^{-1}$ assuming $M = 27 M_{\odot}$ and $R = 20 R_{\odot}$. This value is less than the maximum expansion velocities found in the P Cygni profiles indicating the presence of mass loss similar to that found in other luminous stars¹⁰.

A search was made for variations in the line profiles with binary phase. It can be argued that the structure of the wind changes when the X-ray source is present, either through ionisation of the dominant ion species that drive the wind, or by ionisation of a cavity in the expanding atmosphere of the primary star that would preferentially be in the radial direction.

The two high dispersion short wavelength spectra were taken at phase 0.32 and phase 0.97, the latter when the compact object is eclipsed. Superimposition of the broad profiles of the resonance lines Si IV, N V, and C IV shows effectively the same shape at the two phases in contradiction to some predictions¹³.

The effect of the X-ray source, if any, on the atmosphere of the primary must be confined to a region which is small compared with the extended line-forming region of the resonance transitions. Transitions from excited levels that are formed over a narrow range in velocity may be more responsive to the effects of the X-ray source. An optimum time to obtain spectra may be at phase 0.5 when the compact object is directly in front of the primary star.

The interstellar spectrum is also discussed in the preceding article by Grewing *et al.*¹⁵. It is rich as might be expected from a

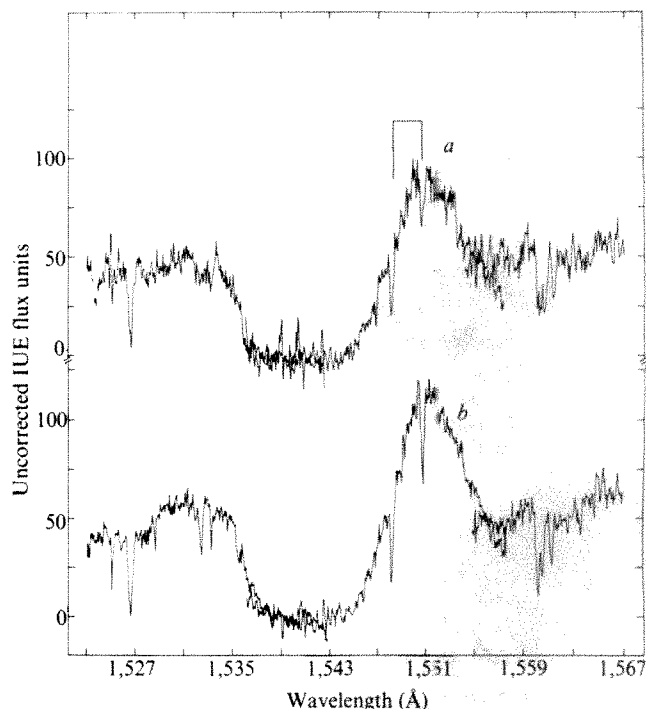


Fig. 1 The region $\lambda\lambda 1523-1567$ of HD153919 (4U1700-37) taken in the high dispersion mode at two orbital phases: *a*, phase 0.32 (SWR 1027); *b*, phase 0.97 (SWR 1054). The broad stellar P Cygni profile of the C IV doublet near $\lambda 1550$ has the same appearance and extent at both phases. The strong narrow features marked at $\lambda 1548$ and $\lambda 1550$ are attributed to interstellar C IV. Other interstellar lines arising from neutral and singly ionised species are apparent; the absorption feature at $\lambda 1526.7$ is most probably interstellar Si II. There was a change of system focus between the two images; the resolution of the lower (phase 0.97) spectrum is superior to the other, as can be noted by inspection of the interstellar profiles. A correction has not been implied for the echelle blaze; the wavelength scale is given directly as inferred from the calibration lamp. The wavelength regions where the orders overlap can be noted by the double tracings; the sharp emission features near $\lambda 1539$ are of instrumental origin.

reddened source with $E(B - V) = 0.52$ (ref. 14) at a distance of 1.2 kpc (ref. 8). Many atomic species C I, Si I, Cl I are present, reflecting a large amount of cold neutral material along the line of sight. The relative weakness of C I lines originating from excited fine-structure levels suggests that the temperature is less than 50 K (J. B. Black, personal communication).

The most notable interstellar features are the strong absorption lines of the highly ionised species of Si IV and C IV (see Figs 1 and 2). The equivalent widths for the C IV doublet at $\lambda 1550.6$ and $\lambda 1548.0$, and the Si IV transition $\lambda 1402.37$ are given in Table 3. Only one of the Si IV doublet transitions is found due to the presence of a strong stellar P Cygni profile that completely absorbs the background stellar continuum. The region near

Table 1 Summary of observations

Star	X-ray source	Camera image	Dispersion	Exposure (min)	Exposure midpoint (JD 244+)	Phase
HD153919	4U1700-37	SWR 1027	High	100	3,552.488	0.32*
		LWP 1029	High	30	3,552.624	0.36
		SWR 1054	High	100	3,564.927	0.97
HZ Her	4U1656+35 (Her X-1)	SWR 1051	Low	180	3,563.290	1.7 day† 0.82
		SWR 1056	Low	240	3,565.276	0.98
		SWP 1272	Low	90	3,598.903	0.76
HDE226868	4U1956+35 (Cyg X-1)	SWR 1050	Low	150	3,562.957	0.48‡
		SWP 1273	Low	40	3,599.049	0.93

* Ephemeris T. Matilsky (personal communication).

† Ephemeris from refs 3-5.

‡ Ephemeris from ref. 6.

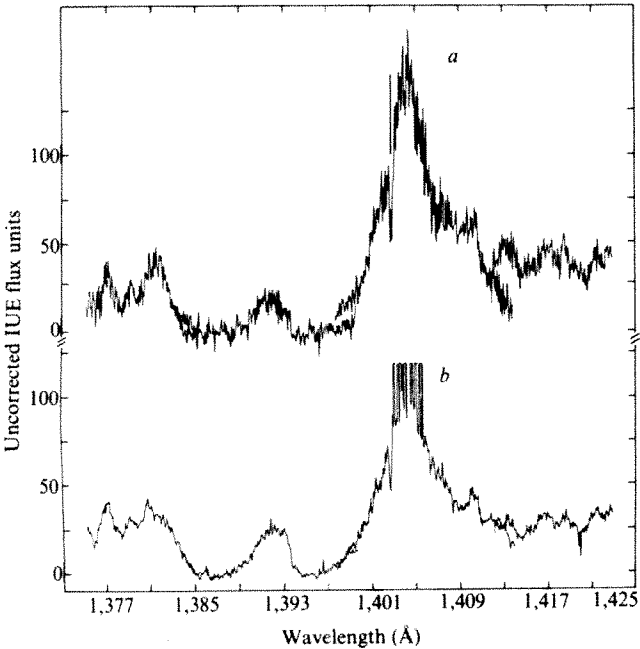


Fig. 2 The region $\lambda\lambda 1377\text{--}1425$ of HD153919 containing the resonance doublet of Si IV at $\lambda 1394$ and $\lambda 1403$. These images were obtained at two orbital phases of the system: *a*, phase 0.32 (SWR 1027); *b*, phase 0.97 (SWR 1054). The focus of the instrument was superior when the lower spectrum was obtained; the emission peak near $\lambda 1405$ is saturated in the lower spectrum. The stellar P Cygni profiles indicate outflow velocities of $\sim 2,500\text{ km s}^{-1}$; there are no substantial differences in the profiles between the two spectra. Interstellar absorption due to Si IV is marked near $\lambda 1403$. See the legend to Fig. 1 for additional notes.

$\lambda 1240$ where interstellar N V occurs was not well exposed in our images, and upper limits to the equivalent widths are estimated from measurements of the peak-to-peak noise over widths of $0.4\text{ \AA}$ at the expected position of the lines.

The lines are assumed to lie on the linear part of the curve of growth. For Si IV, in which one line is found, this assumption gives a lower limit to the column density. The C IV transitions have central intensities which are about half of the continuum intensity; moreover their equivalent widths are in the expected 2:1 ratio confirming the assumption of unsaturated lines. The derived column densities (see Table 3) are substantially higher than those found for ζ Oph, a cooler less luminous star of spectral type O9.5 V. Highly ionised species are about twice as abundant towards HD153919 than towards ζ Pup, an O4 If star of comparable spectral type. HD153919 is at a distance of 1.2 kpc which is about 2.5 times the distance of 450 pc to ζ Pup so that we cannot rule out a large scale distribution of C IV and Si IV. However, if these ion species are confined to an H II region near the star, their higher column density may reflect the

Table 2 Velocities (km s^{-1}) of P Cygni profiles in HD153919 (Phase 0.97)

Ion	λ_{lab} ($\text{\AA}$)	Emission maximum	Absorption centre	Short edge
He II	1,640.33	−3	−420	−640
C III	1,175.7	+580	−1,400	−2,200
C IV	1,550.77 1,549.06* 1,548.18	+87†	−1,500	−2,600
N IV	1,718.55	+320	−1,000	$\approx$ −1,800
N V	1,242.80 1,240.15* 1,238.82	+200	−1,400	−2,300
O IV	1,338.61	+230	−220	−440
Si IV	1,402.77 1,393.76	+270 —	−1,600 −1,500	−2,100 −2,600

* Mean wavelength for unresolved multiplet.

† This maximum may appear at shorter wavelengths due to the (uncorrected) blaze of the grating.

Table 3 Highly ionised interstellar species

Ion	$\lambda(\text{\AA})$	$W(\text{m\AA})$	f	$\log N(\text{cm}^{-2})$		
				HD153919 observed	HD153919 predicted*	ζ Pup† ζ Oph†
C IV	1,550.77	112	0.097	13.71	14.3	13.37 <12.7
	1,548.18	213	0.194	13.72		
N V	1,238.82	<133	0.152	<13.81	13.7	<12.2 <12.3
	1,242.80	<56	0.076	<13.74		
Si IV	1,402.77	104	0.262	≥ 13.36	14.2	13.1 12.7

* Ref. 16.

† Ref. 12.

‡ Ref. 17.

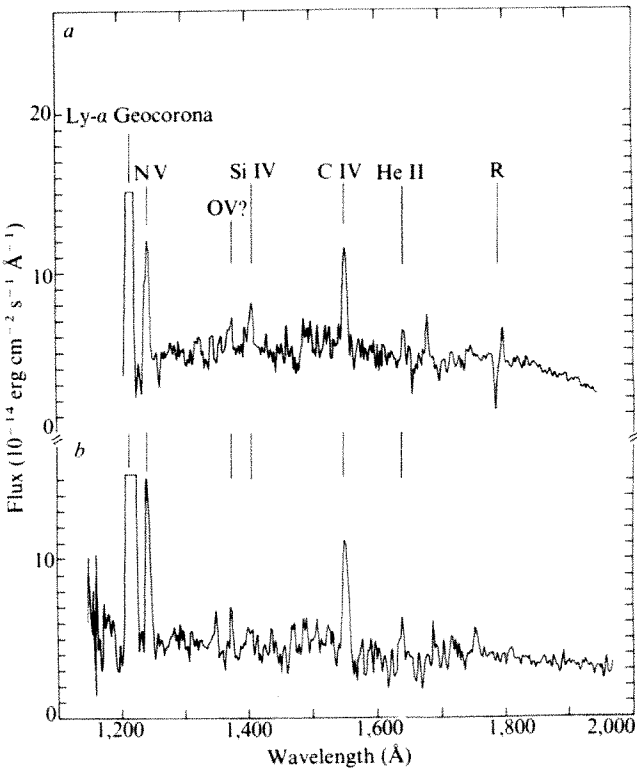
effects of ionisation by the X-ray source, although the observed column densities are still considerably less than those predicted by McCray *et al.*¹⁶ for HD153919.

Relative abundances of ion species may be used to discriminate between various sources of ionisation. In Table 4, we list the abundances of N V and C IV relative to Si IV that are observed towards HD153919 and towards ζ Oph and ζ Pup. These values are compared to calculations for a model of HD153919 immersed in the photoionised interstellar gas¹⁶, and for an interstellar bubble that can be caused by a strong stellar wind¹⁸. In the former case, a relatively cool but highly ionised gas can result from the EUV and X-ray radiation from HD153919. The bubble produces a conduction-dominated interface between the hot shocked (10^6 K) gas and the cold interstellar medium. This interface is the source of the absorption lines. The relative abundances that are observed (Table 4) are substantially less than those estimated for an interstellar bubble. The observations seem to support the presence of a photoionised gas near both the X-ray source and the two hot stars.

HZ Her (Her X-1)

The eclipsing binary system HZ Her contains a primary star that varies between spectral types A and F, and one of the first pulsating X-ray sources to be identified¹⁹. Periodic flux varia-

Fig. 3 The short wavelength spectrum of HZ Her at low dispersion ($\sim 6\text{ \AA}$): *a*, phase 0.76; *b*, phase 0.82. Several emission lines are apparent at both phases: N V; Si IV; C IV; and He II. The feature marked R results from a fiducial mark (reseau) on the faceplate of the camera tube.



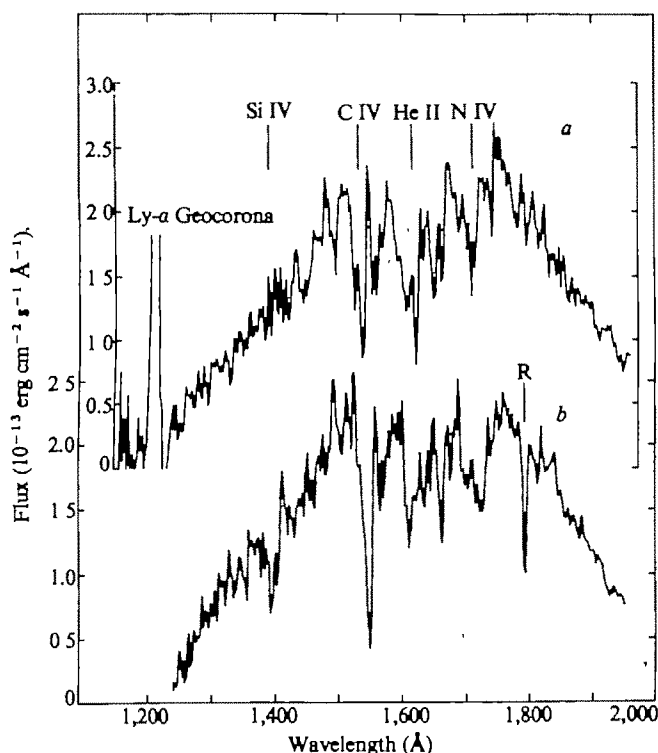


Fig. 4 The short wavelength spectrum of HDE226868 (Cyg X-1) at two phases: a, phase 0.48, b, phase 0.93. Several strong absorption lines are noted. The feature marked R near $\lambda 1800$ results from a rescan mark.

tions are present at optical and X-ray wavelengths resulting from the binary motion of the system (1.7-d period), the rotation of the compact object and perhaps X-ray shadowing of the accretion disk²⁰ (35-d X-ray on/off period, and associated optical variability). X-ray heating of the primary star, mass accretion by the compact object, and variable absorption^{21,22} cause additional fluctuations in measured emissions.

The spectrum of HZ Her from $\lambda\lambda 1200$ –2000 was obtained at three phases during the X-ray on period of its 35-d cycle (see Table 1). Emission lines attributed to N V, Si IV, C IV and He II are clearly apparent in exposures taken at orbital phase 0.82 and 0.76 (see Fig. 3). However, a 4-h exposure centred at phase 0.98 (eclipse of the compact object) showed no line emission except for the geocoronal Ly α , suggesting that the source of UV emission is restricted in size and is located in the region on or about the opposing side of the primary, facing the compact object. An upper limit for the line and continuous flux between $\lambda 1200$ –2000 at phase 0.98 is estimated to be 1×10^{-14} erg cm $^{-2}$ s $^{-1}$ Å $^{-1}$.

The N V emission is surprisingly strong relative to C IV. Calculations²³ have been made of the line emission from a spherical, gas cloud of uniform density that is photoionised by a central X-ray source. The calculations suggest that generally C IV should be about six times stronger than N V, while our observations suggest nearly equal emission fluxes.

There is an Fe XII line at $\lambda 1242$ which could be blended with the N V doublet at $\lambda 1240$. If so, another Fe XII line at $\lambda 1350$ should be present although weaker than $\lambda 1242$, by a factor of ~ 3 in the high density limit. A feature at $\lambda 1350$ is apparent in both spectra of HZ Her (Fig. 3); if this line is indeed Fe XII, then the contribution of Fe XII at $\lambda 1242$ to the N V blend could be significant in reducing the inferred intensity of N V.

The continuum has an approximate spectral index $F_\nu \propto \nu^{-1.3}$, and is presumably due to the heating of the primary atmosphere by X rays. This is apparently in general agreement with the calculations of Milgrom and Salpeter²⁵, although their results are presented in wide bandpasses, making direct comparison difficult. The continuum flux at $\sim 1,500$ Å is $\sim 20\%$ higher at phase 0.76 than at phase 0.82; this is a marginal result, due to the

Table 4 Relative abundances of highly ionised interstellar species

Source	N(C IV)/N(Si IV)	N(N V)/N(Si IV)
HD153919 (observed)	<2.3	<2.5
ζ Oph	<0.92	<0.38
ζ Pup	1.5–2.3	<0.08
Photoionised volume ¹⁶	1.2	0.3
Interstellar bubble ¹⁸	15.9	6.3

inherent errors and the fact that for operational reasons two different cameras were used. Milgrom and Salpeter²⁵ predict a factor of two change in the continuum between these phases, which may be ruled out.

HDE226868 (Cyg X-1)

The binary system HDE226868 is identified with the X-ray source Cyg X-1. The primary star is classified²⁶ as O9.7Iab, and the X-ray producing secondary is perhaps the strongest candidate for a black hole of any compact X-ray source.

The low dispersion spectrum of HDE226868 is shown in Fig. 4 at two phases. The spectrum from the supergiant has been detected and is generally typical of a highly reddened star. This star shows broad absorption features most probably of C IV ($\lambda 1550$), He II ($\lambda 1640$), and N IV ($\lambda 1718$). The absorption, particularly of C IV, seems to be asymmetric and indicative of mass loss. Strong P Cygni emission components as found in stars of similar type from Copernicus¹⁰ are absent. There is apparently a substantial phase effect in this spectrum with the strengthening of the Si IV and C IV transitions near superior conjunction when the supergiant lies between the Earth and the compact object. These features may be similar to the absorption dips reported in soft and hard X-ray observations that occur preferentially near superior conjunction, and reflect the presence of matter between the binary components^{28–30}. Other lines such as the feature near $\lambda 1710$ may appear at phase 0.93. We should point out that the second spectrum was taken with a different camera and a better focus, so that the depth of the features could be affected.

The strength of Si IV in early-type stars seems to be a strong function of luminosity class. The equivalent width of the Si IV doublet at phase 0.93 is 6 Å, which is less than the O9.5 Ia star α Cam, but clearly more than the O9.5 V star δ Ori A (ref. 31). Thus the strength is compatible with a high luminosity for the primary.

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IUE observations of extragalactic objects

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During the commissioning phase of IUE several extragalactic objects were observed spectrally at low dispersion in the UV range $\lambda\lambda 1150\text{--}3200$: the Seyfert galaxies NGC4151 and NGC1068, the QSO 3C273, the BL Lacertae object B2 1101+38, the giant elliptical galaxy M87 and the spiral galaxy M81. The results obtained are presented and a preliminary analysis given for all six objects, discussing the continuous spectrum, extinction, emission line spectrum and absorption line spectrum, where possible for each case. Several new or confirmatory astrophysical results are obtained.

DURING the commissioning high priority target phase of IUE several classes of extragalactic object were observed at low dispersion with both the short- and long-wavelength cameras. Although the instrument was not fully optimised, scientifically useful spectra were obtained for the Seyfert galaxies NGC4151 and NGC1068, the QSO 3C273, the BL Lacertae object B2 1101+38, the giant elliptical galaxy M87 and the spiral galaxy M81. A journal of observations is given in Table 1, which includes some related optical photometry (performed by A.S.). Figure 1 gives photographic reproductions of the short wavelength spectra of four of the objects observed. All six objects are discussed separately in following sections, and are preceded by a description of the adopted calibration, which differs in detail from that used for most of the other high priority targets discussed in the accompanying articles.

Absolute calibration

The initial absolute calibration of the scientific instrument is discussed by Boggess *et al.*¹ This is based on the OAOII scale² which differs from all other recent calibrations in the region between 1,350 and 1,700 Å, having a maximum departure of about 30% at $\lambda 1550$ (refs 3, 4). Extragalactic data reduced with the absolute calibration based on the OAOII scale show a hump

in the continuum around $\lambda 1550$. The same hump has been noted in the flux distribution of hot stars observed by IUE when reduced to the OAOII flux scale^{5,1}.

We have carried out further work on the absolute calibration because this is critical for establishing the slopes of the non-thermal continua. First, we linked the calibration mainly to the TD-1 (S2/68) fluxes, supplemented by ANS data. Second, we used additional objects to HD60753, the one used by Boggess *et al.*¹ This was accomplished without additional observations as some of the high priority objects, observed during the same period as the extragalactic objects presented here, are excellent calibration standards themselves. We used the following as additional calibration objects: BD+75 325, which was compared with OAOII data⁶, and data from ANS and TD1 S2/68 (P. R. Wesselius and D. J. Carnochan respectively, personal communications); HZ43, for which ANS data are available⁹; and HD137389, which has been observed by the S2/68 instrument¹⁰. The calibration for the stars listed by Boggess *et al.*¹ was converted to the S2/68 scale using the correction factors given by Beeckmans³.

For the SWR camera, calibration curves obtained from the individual stars were averaged, giving double weight to the results for BD+75 325 and HZ43. The mean calibration curve so obtained was used for the reduction of all SWR extragalactic images. When compared with that given by Boggess *et al.*¹ this calibration curve shows two differences: the sensitivity minimum around $\lambda 1550$ is less pronounced, reflecting the difference between the OAOII and S2/68 or ANS flux scale (our continuum fluxes no longer have the $\lambda 1550$ hump), and the sensitivity at all wavelengths is lower. The extent of the second is comparable with the difference found by Boggess *et al.*¹ between the sensitivities obtained, after camera reoptimisation, for μ Col and HD60753. The four stars analysed for the SWR calibration all give roughly the same wavelength dependence for the sensitivity but the individual stars give absolute calibrations different by up to $\pm 20\%$ from the mean curve. Thus, the slope of a spectrum is well defined, but the overall flux level could be in error by $\pm 20\%$, in addition to any errors in the OAOII, S2/68

Table 1 Journal of observations

Date (1978)	Object	Camera,* band	Exposure (min)	Comments
11 February	NGC4151	SWR	60	Partly out of aperture; poor focus
11 February	NGC4151	LWP	120	
17 February	M87	LWP	180	
22 February	M81	SWR	120	
22 February	NGC1068	SWR	180	Good focus
27 February	3C273	SWR	180	
27 February	3C273	LWR	150	
27 February	3C273	SWR	60	
28 February	B2 1101 + 38	LWR	77	High radiation level
28 February	B2 1101 + 38	LWR	100	
28 February	B2 1101 + 38	SWR	240	
28 February	NGC4151	SWR	120	Good focus
28 February	B2 1101 + 38	UBVI		
7 March	NGC4151	UBV		
7 March	3C273	UBV		

*IUE (10" × 2" aperture, low dispersion):

SWR (short wavelength redundant camera)

LWR (long wavelength redundant camera)

LWP (long wavelength prime camera)

Optical Photometry (Rosemary Hill Observatory, University of Florida):

UBVI

UBV.

or ANS calibrations and the errors involved in the transformation of the IUE data to other calibration systems (the latter is estimated to be ~10% for well-exposed spectra¹).

For the long wavelength cameras the high priority stellar targets permitted only a rough check for $2,000 \leq \lambda \leq 2,540$. For the accuracy of the measurements the results agree with the standard calibration¹, and this was used for the reduction of the objects in this paper.

Finally, we note that the 20% error mentioned above applies to both the short- and long-wavelength cameras, and the present results for the interstellar absorption maximum near $\lambda 2200$, which has to be measured on both the long and the short wavelength spectra for objects of low redshift, and is also subject to extra noise and uncertain end effects, should be treated as preliminary only.

NGC4151

Comprehensive reviews of observational results and model computations for Seyfert galaxies are given by Weedman¹¹ and Collin-Souffrin⁶⁶. The well-known Seyfert galaxy NGC4151 is the brightest of the class with broad permitted emission lines (Class 1 of Khachikyan and Weedman¹²) in the northern skies and has been closely studied at all available wavelengths. The permitted lines also contain a distinct narrow component with the same profile as the forbidden lines (which are always relatively narrow in Seyfert galaxies) and NGC4151 is better classified 1.5. Its nucleus contains a compact source which is variable at X-ray, UV, optical and IR wavelengths^{13-17,49}. It is also a γ -ray source¹⁸ and a weak source of radio emission¹⁹, although the radio emitting volume may be different from the region producing the optical continuum and broad emission lines²⁰. Recently an UV spectrum of NGC4151 has been obtained by Davidsen and Hartig with a rocket-borne telescope⁴⁶.

The optical spectrum of NGC4151 has been discussed by several authors²¹⁻²⁵. The narrow-line region has temperature $T = (11,750 \pm 800)\text{K}$, and electron density $N_e = 2 \times 10^4 \text{ cm}^{-3}$ in a higher ionisation region and $N_e = 5 \times 10^3 \text{ cm}^{-3}$ in a lower ionisation zone defined by [O I], [O II] and [S II], which will be excited at the outer portions of the gas²⁴. The relative intensities of the forbidden lines can be well matched by photo-ionisation models in which the gas is ionised by a non-thermal UV continuum, and probably this is also the main energy source for the broad-line region^{26,27}. The broad-line region is characterised by the permitted emission lines of H I, He I, He II and Fe II, and must have $N_e \geq 10^8 \text{ cm}^{-3}$ to collisionally de-excite the forbidden lines which are absent^{28,24}. High column densities are also indicated by the Balmer decrement, which can be understood

only if the higher Balmer lines are optically thick^{22,23,39}. As is commonly observed in such objects, the broad components of the Balmer lines in NGC4151 are asymmetric and extend over several thousand km s^{-1} ; the He II $\lambda 4686$ profile is similar, but He I $\lambda \lambda 5876$ and 7065 are much broader²⁴. The asymmetries and different linewidths rule out electron scattering as the dominant broadening mechanism, and invalidate the model for the line emitting region developed by Ptak and Stoner^{29,24}. There is now little doubt that the broadening is chiefly due to mass motions of the ionised gas in such active nuclei^{27,30,31}.

A striking feature of the optical spectrum of NGC4151 is the presence of non-stellar Balmer and He I $\lambda 3889$ absorption features which are blueshifted with respect to the emission lines and variable in time^{32,33,25,140}. These features are characteristically different from the absorption lines generally present in QSO spectra, which arise only from ground-state transitions and have never been seen to vary. Galactic interstellar Ca II absorption is also present in the spectrum of NGC4151 (refs 24, 25, 140).

NGC4151 was observed by IUE with both the short and long wavelength cameras (Table 1). The better exposed of the two short wavelength spectra (28 February) is shown in Fig. 2. Emission features (28 February for short wavelength, 11 February for long) are listed in Table 2 and the absorption features (28 February) in Table 3. Optical spectra taken soon after (8 March) (J. McClintock, personal communication) show that the visual absorption lines were particularly strong. For the absorption lines arising from levels of the ground state there is probably a significant galactic interstellar contribution in some cases (the heliocentric velocity of NGC4151 determined from the emission lines is about 950 km s^{-1} (ref. 32), and the absorption lines are blueshifted relative to NGC4151 by a comparable amount^{32,140}, bringing them to near-zero velocity in the observed frame).

The continuous spectrum of NGC4151 obtained on 28 February, with measurements averaged over bands 20–100 Å wide avoiding the emission and absorption lines, is shown in Fig. 3. We include our UBVI photometry (7 March), corrected for the emission lines and Balmer-continuum emission (the latter from the expression given by Stein and Weedman³⁴) and reduced to the same effective aperture as the IUE observations (10" × 20") from the data of Penston *et al.*¹⁶. We also show the continuum data of Davidsen and Hartig⁴⁶, obtained on 10 February.

The observed optical continuum of an active galaxy nucleus is made up of two parts: the featureless non-thermal or 'power law' continuum unique to the active nucleus, and the integrated galaxy continuum (containing absorption lines) due to stars in or near the nucleus. (The term 'non-thermal' is used rather loosely; 'non-stellar' might be better in some cases where a thermal source, possibly complex, cannot be ruled out.) When the continuum shows variability the spectral slope generally also varies due to the differing contributions of the non-thermal variable component and the stellar component, although the slope of the non-thermal component probably does not change¹⁴. Assuming a power-law spectrum of form $F_\nu \propto \nu^{-\alpha}$ we

Fig. 1 Photographic reproductions of unprocessed short wavelength spectra of four of the extragalactic objects: first, the Seyfert 1.5 galaxy NGC4151; second the Seyfert 2 galaxy NGC1068; third the QSO 3C273; fourth the BL Lac object B2 1101 + 38 (Markarian 421). Wavelength increases to the right. The large emission feature at the left of each spectrum is geocoronal Ly α which fills the 10 × 20 arc s entrance aperture.

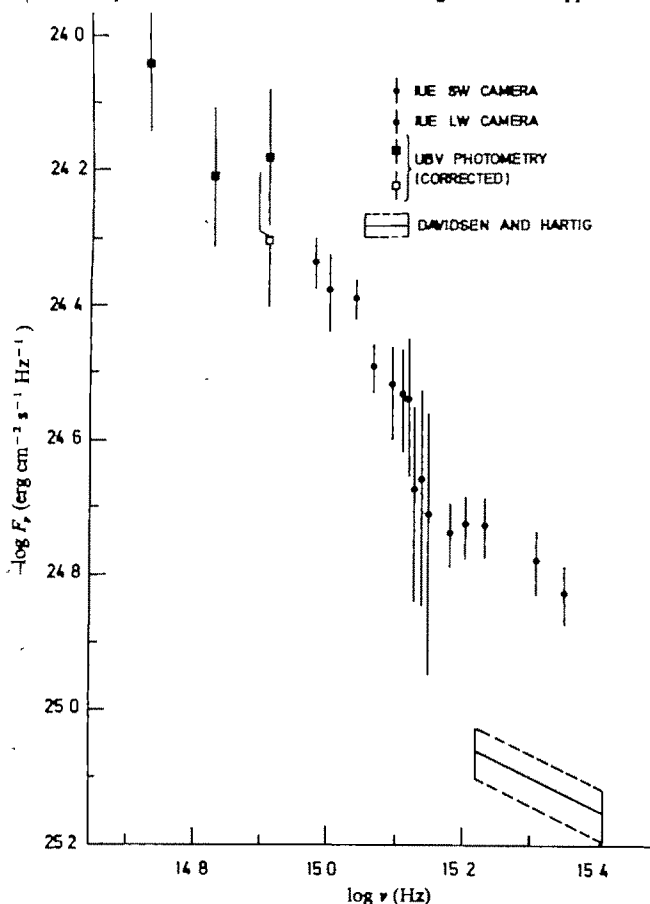


The different degrees of reddening obtained for the continuum and forbidden line regions is interesting because it shows that our view of the continuum source is relatively unobscured by line-emitting gas, which therefore does not totally surround the continuum source. However, a covering factor as little as 0.7 is consistent with the observed strength of H β and our far UV continuum slope. A model of the ionised gas in Seyfert nuclei deduced from observational data is given by Osterbrock²⁷. We return to this later.

The spectrum of NGC4151 shows strong emission lines of Ly α , C IV λ 1550, He II λ 1640, C III] λ 1909, [O III]+C II] λ 2324, [Ne IV] λ 2424 and Mg II λ 2800, and several weaker lines (Fig. 2 and Table 2). There is little evidence of N V λ 1240, Si II λ 1263, O I λ 1305, C II λ 1335 or Si IV+O IV] λ 1400 in emission, but for these the spectrum is confused by definite or possible absorption features. Nevertheless, N V λ 1240, if present, is very weak. The presence of [Fe XI] λ 2650 is consistent with the detection of the coronal line [Fe XI] λ 7892 in this object⁴⁵.

The Ly α profile is affected by the geocoronal emission, a fiducial mark (reseau) and Ly α and N V absorption, but it seems to have comparable width to the Balmer lines²⁴. The C IV profile also is strongly affected by absorption, which makes it difficult to determine the FWHM, but it seems peculiarly broad with very extensive wings. Accounting for contributions from N IV] λ 1486, [Ne IV] λ 1602 and possibly [Ne V] λ 1575 still leaves a very broad asymmetric line of full width at zero intensity $\approx 15,000$ km s⁻¹, which is comparable with the broad He I lines observed in the visible²⁴ and suggests a common origin. Alternatively, there may be a strong contribution from Fe III to the C IV profile, as contended by Davidsen and Hartig⁴⁶, but

Fig. 3 NGC4151 continuum fluxes (28 February 1978 for SW, 11 February 1978 for LW, see Table 1). The IUE data are averaged over bands 20–100 Å avoiding the emission lines. Our UVB photometry (6 March 1978) reduced to the IUE observing aperture, also are corrected for emission lines (■) and additionally for Balmer continuum emission (□). The continuum data of Davidsen and Hartig⁴⁶, obtained on 10 February 1978, are included. The error bars indicate 1 σ statistical uncertainties in signal only, and do not include possible calibration errors. No reddening correction is applied.



according to the results of Viotti⁴⁷ Fe III emission around λ 1550 should be accompanied by even stronger Fe III emission between $\lambda\lambda$ 1840 and 2020, which is absent in our data. In contrast, C III] λ 1909 is relatively narrow, but a broad component of comparable width to that of the Balmer lines is clearly present, blended with Si III] λ 1892 in the blue wing. This sets an upper limit of $N_e \leq 10^{10}$ cm⁻³ to the broad-line region.

Netzer³⁹ has shown that a steep Balmer decrement can be produced if $N_e > 10^8$ cm⁻³, in explanation of observed inconsistencies when attempting to fit extinction-corrected Balmer line strengths for QSO and Seyfert galaxies to values predicted from radiative recombination theory. Baldwin⁴⁰ has pointed out a more extreme difficulty for the intensity ratio of Ly α to H β in QSOs, which is directly observed or deduced to be ≈ 3 (refs 40–42). Recombination theory predicts values 22–40 depending on the extent that Ly α is collisionally excited. While selective extinction by dust could reduce the relative intensity of Ly α , particularly if it has a large optical depth compared to H β and is multiply scattered, it is curious that $I(\text{Ly}\alpha)/I(\text{H}\beta)$ is so remarkably constant among cases having substantially different apparent reddening. This matter has been further discussed recently by Zirin⁴⁰. For the Seyfert galaxy NGC4151, also, we find $I(\text{Ly}\alpha)/I(\text{H}\beta) = 4.4 \pm 2$ using $I(\text{H}\beta) = 6.6 \times 10^{-12}$ erg cm⁻² s⁻¹ (refs 21, 22, 24). Assuming that $E(B-V) = 0.6$ holds not only for the continuum but also for the permitted line region (which emits most of the flux in the hydrogen lines), and making a simple application of the same extinction law as before, gives $I(\text{Ly}\alpha)/I(\text{H}\beta) = 6.3 \pm 3$. On the other hand, if $E(B-V) = 0.24$, as deduced for the narrow-line region from the [S II] lines, we obtain $I(\text{Ly}\alpha)/I(\text{H}\beta) = 18 \pm 8$, closer to the recombination value. Note that the presence of geocoronal Ly α , Ly α absorption in the Galaxy and in NGC4151, and the camera fiducial mark in the red wing of the NGC4151 Ly α line makes the measurement of this line rather uncertain.

Baldwin⁴³ has found that for QSOs there exists an important correlation between $W_{\lambda_0}(\text{C IV})$, the rest frame equivalent width of the C IV λ 1549 emission doublet, and $L_{\nu}(1450)$, the continuum luminosity at a rest wavelength λ 1450 computed on the assumption that the redshift is a cosmological indicator of distance, suggesting that these objects can be used as standard candles. The relationship seems particularly tight if restricted only to the flat spectrum radio sources, as confirmed by Baldwin *et al.*⁴⁸. The similar study by Omer⁴⁴ is in apparent conflict, showing $W_{\lambda_0}(\text{C IV})$ to be independent of $L_{\nu}(1475)$, but he covered a smaller luminosity range and his results are consistent with Baldwin's more widely ranging data. Comparing our (rather uncertain) value of $W_{\lambda_0}(\text{C IV})$ for NGC4151 with

Table 3 Absorption lines in the spectrum of NGC4151 ($\lambda < 2,000$, 28 February spectrum; $\lambda > 2,000$, 11 February spectrum)

Identification	λ_0 (Å)	W_{λ_0} (Å)	Comments
C III*	1,176	4†	
Si II	1,193	1†	
N V	1,239, 1,243	6†	
Si II	1,260	—	Weak
Si II*	1,265	—	Weak
O I	1,302	—	Possible
C II, C II*	1,335, 1,336	3†	Seems diffuse
Si IV	1,394	3.5†	
Si IV	1,403	1.5†	
?	1,425*	1†	Possible
?	1,456*	1†	Possible
?	1,473*	—	Possible
C IV	1,548, 1,551	>3	In strong emission line
Al III	1,855	1†	
Al III	1,863	1†	
?	1,963*	1†	Possible
Fe II	2,587–2,632	4†	Broad shallow feature
Mg II	2,796, 2,803	$2 < W_{\lambda_0} \leq 6$	Lean strong emission line; probably partly galactic

*Wavelength measured relative to other known lines.

†The unknown contribution of the emission lines to the local 'continuum' for the absorption lines makes the values of equivalent width for these uncertain.

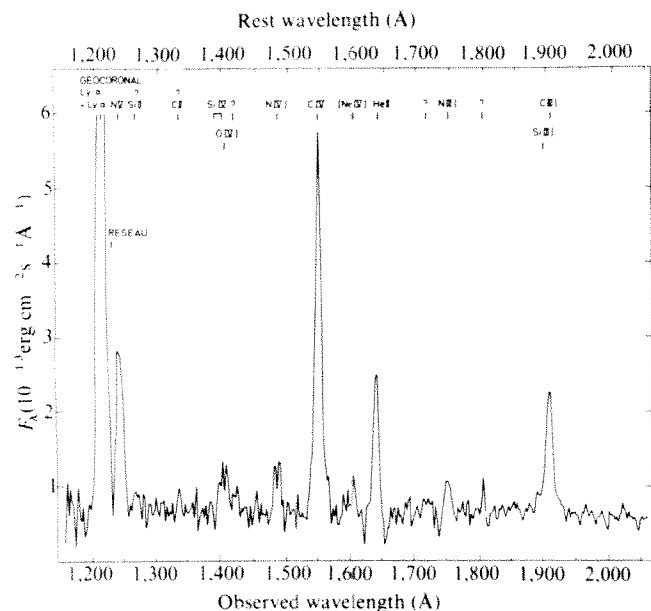


Fig. 4 Short wavelength spectrum of the Seyfert 2 galaxy NGC1068, photometrically corrected and calibrated. Emission lines are indicated, including suspect and apparent unidentified features. The Ly α line is saturated, and contaminated by geocoronal Ly α . The almost complete separation of Ly α and NV is real, not due to the fortuitous presence of the reseau (see Fig. 1).

Baldwin's relationship gives an expected luminosity $\log L_v(1450) = 30.1$, but we find the value 27.9, which is in serious conflict. Later we shall show the Seyfert galaxy NGC1068 and the BL Lac object B2 1101 + 38 (which is a flat spectrum radio source) also do not fit the relationship but the QSO 3C273 does fit well. We conclude that Baldwin's luminosity relationship at least is not generally applicable to active nuclei, and with the available evidence seems to segregate QSOs from Seyfert galaxies and (less surprisingly) BL Lac objects. However, excluding B2 1101 + 38, we note that over the large luminosity range now obtaining with the inclusion of NGC4151 and NGC1068 with the sample of QSOs, the data broadly conform with Osmer's⁴⁴ results which show no luminosity dependence for W_{λ_0} (C IV).

From model computations Shields⁵¹ has shown that use of some of the weaker emission lines allows abundance determinations that are not strongly dependent on the details of the photoionisation models for active nuclei; in particular, these are: O III] λ 1663, N IV] λ 1486, N III] λ 1749 and C III] λ 1909. Baldwin and Netzer⁵² made a refined analysis and find some minor modifications to Shield's results. Using Shield's relationships we find the relative abundance N/C is 0.16 from N IV]/C IV and 0.13 from N III]/C III]. Baldwin and Netzer⁵² find N III]/C III] is ~ 1.5 larger in their analysis, and using their correction we obtain N/C = 0.09. For O/C and N/O we obtain 1.37 and 0.20 from use of O III]/C IV and N IV]/O III] respectively. Given the uncertainties in our line fluxes, these values are reasonably self-consistent and give relative abundances N/C/O which are comparable with solar values¹⁴¹.

Our two short wavelength spectra were obtained 17 d apart (Table 1). Although the 11 February exposure is somewhat out of focus and underexposed, there is clear absorption present at C IV λ 1550, Si IV $\lambda\lambda$ 1394, 1403 and C II $\lambda\lambda$ 1335, 1336. If N V λ 1240 is present it is rather weak. Our well-exposed and in-focus spectrum of 28 February shows the several lines listed in Table 3. N V is strong, C II seems weaker, and C IV stronger with the absorption more centrally placed in the emission line, than on 11 February. C II seems rather diffuse (28 February spectrum), contrasting with the sharp Si IV and Si II lines. Time-varying structural differences between the He I λ 3888 and Balmer line absorption features have been observed in the optical spectrum^{141,32}. In the spectrum obtained by Davidsen and Hartig⁴⁶ one day earlier than our first spectrum, N V, Si IV

and C IV seem absent, Si II and C II are strong, and there may be other features. Their resolution is lower than ours, and C IV, for example, may be present but lost in the strong emission profile. However, at face value there is evidence that the ionisation of the absorption system has changed greatly over the period of 18 d between the observations of Davidsen and Hartig and our good 28 February spectrum, and to some degree in the one day between their observation and ours of 11 February. Some change in line strength is evident over 17 d from the IUE data alone. The dramatic increase in ionisation apparent from the time of Davidsen and Hartig's observations is accompanied by a large increase in the far UV continuum flux discussed earlier, if real.

The presence of the C III* λ 1176 absorption line, which arises from the metastable $2p^3P^0$ state, is consistent with the excitation of the optical spectrum^{25,32,140}. Si II* λ 1265 arises from an excited fine structure level, and seems comparable in strength to the ground state line λ 1260, suggesting that the electron density in the absorbing region is $N_e > 10^4 \text{ cm}^{-3}$ if the level is populated by collisions at $T_e \approx 10^4 \text{ K}$. However, the Si II lines are only marginally detected.

At our relatively low resolution we cannot determine the true depth of the absorption lines, but it is clear from the observed depths of the various strong absorption features that not only the continuum but also a substantial fraction of the broad-line-emitting region is covered by the absorber. Thus, the absorbing region cannot be a component (filament) of the broad-line-emitting region but must be more extensive and outside it. A similar conclusion was reached by Anderson³² from the optical data.

NGC1068

NGC1068 is the archetype of class 2 Seyfert galaxies, and has been well studied at optical, IR and radio wavelengths^{16,20,54-63,68}; it shows no convincing variability. It has an IR nucleus $\sim 100 \text{ pc}$ in diameter, which is inconsistent with a single non-thermal source, but is consistent with emission from heated dust⁵⁸. Emission features due to H₂ are present in the IR spectrum⁶², and the absorption feature near $9.5 \mu\text{m}$ which has been attributed to silicates is strong⁶⁴. It has somewhat broader lines than other Seyfert 2 galaxies, with FWHM about $1,200 \text{ km s}^{-1}$, but they are still considerably narrower than in Seyfert 1 galaxies. The lines have an asymmetric form with much velocity structure, and spatial structure discernible on a scale of $1''$ or 70 pc (ref. 65) and extending over $\sim 8''$ (ref. 59). The line emitting region is physically complicated, having regions of different density, filling factor and degree of shadowing from the continuum source^{57,56}. NGC1068 is another of several Seyfert galaxies found to emit the coronal line [Fe XI] λ 7892 (ref. 45).

NGC1068 was observed by IUE only with the short wavelength camera. The spectrum is shown in Fig. 4. Only emission features are definitely observed, and these are listed in Table 4. The spectrum was over-exposed in Ly α , and this line

Table 4 Emission lines in the spectrum of NGC1068

Identification	λ_0 (Å)	W_{λ_0} (Å)	F ($10^{-13} \text{ erg cm}^{-2} \text{ s}^{-1}$)	Comments
N V	1,240	43	29	
Si II	1,263	4†	3†	Possible
C II	1,335	3†	2†	Possible
Si IV + O IV]	1,400	13	9	
?	1,420*	4†	3†	Possible
N IV]	1,486	11	7	
C IV	1,550	90	59	
[Ne IV]	1,602	5†	3†	
He II	1,640	28	18	
?	1,720*	7	4	Probable, maybe N IV
N III]	1,749	6	4	
?	1,800*	—	—	Possible
Si III]	1,892	—	—	
C III]	1,909	40	25	

*Wavelength measured relative to other known lines

†Uncertain.

also is contaminated by the geocoronal emission, so we have no reliable flux estimate. The almost complete separation of Ly α and N V λ 1240 is real, not due to the fortuitous presence of the *resseau* (see Fig. 1).

Continuum fluxes, mostly averaged over about 40Å and avoiding the emission lines, are shown in Fig. 5, together with part of Wampler's⁵³ spectrophotometry also avoiding emission lines and reduced to the IUE observing aperture with the aid of the data of Penston *et al.*¹⁶. We find $\alpha = 2.4$ fitted to the usual power law as a mean over the whole optical and UV region included in Fig. 5. With our aperture, the visual flux of the nuclear source is much weaker than that of the underlying galaxy^{16,69} but in the far UV (IUE) region, where contamination from the galactic component is negligible, we obtain the important result that the continuum clearly is a power-law spectrum, with $\alpha \approx 1.8$ (not accounting for reddening); there is no unambiguous evidence of a power-law continuum in the optical region. We have no direct estimate of reddening from the IUE data (the region containing the λ 2200 absorption feature was not observed) but if we apply a correction based on the value $E(B-V) = 0.5$ derived from the [S II] lines and the Balmer decrement^{55,67} the resulting spectrum is highly inconsistent with a power law continuum, suggesting that $E(B-V)$ for the continuum is much smaller (≤ 0.15) and implying $\alpha \geq 1.4$. However, a flatter continuum than observed may be needed to explain the presence of [Fe XI] (ref. 45) and the observed value of $I(\text{He}^+)/I(\text{H}^0)$ (ref. 57).

The different degrees of reddening deduced for the continuum and emission line regions again shows that the continuum is not completely surrounded by line-emitting gas. This is consistent with optical and radio data^{57,61}.

The spectrum of NGC1068 shows strong, relatively narrow, emission lines of Ly α , N V λ 1240, C IV λ 1550, He II λ 1640 and C III] λ 1908 (Fig. 4 and Table 4). Several weaker lines are definitely present, including Si IV + O IV] λ 1400, N IV] λ 1486 and N III] λ 1749.

Comparing our value of W_{λ_0} (C IV) for NGC1068 with Baldwin's⁴³ empirical relationship coupling this quantity with

Fig. 5 NGC1068 continuum fluxes. The IUE data are averaged over about 40Å bands. Wampler's⁵³ spectrophotometry is reduced to the IUE observing aperture. Both sets of data are chosen to avoid emission lines. The error bars indicate 1 σ statistical uncertainties only. No reddening correction is applied.

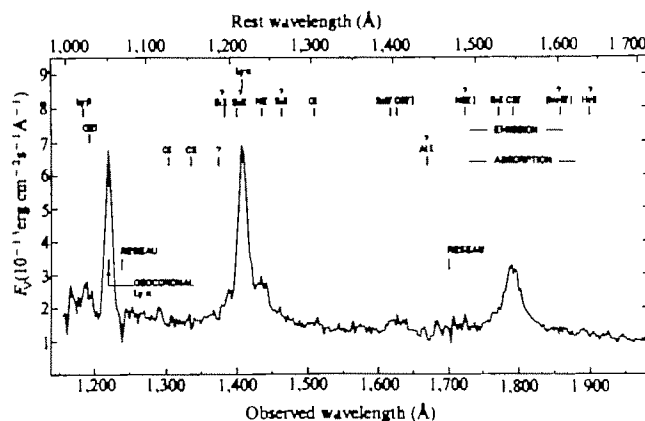
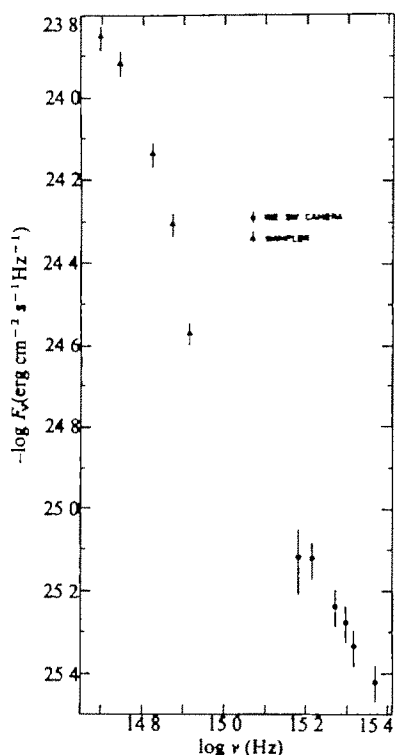


Fig. 6. Short wavelength spectrum of the QSO 3C273, photometrically corrected and calibrated. Emission and absorption lines are indicated (see related comments in the caption to Fig. 2)

$L_p(1450)$, as we have done for NGC4151, gives an expected luminosity $\log L_p(1450) = 30.1$, but we again find a much lower value, 27.4. Thus, we find serious (and similar) departures from Baldwin's relationship for both the Seyfert galaxies studied here.

Calculating the relative abundance N/C from the work of Shields⁵¹ and Baldwin and Netzer⁵², as before, we find the value ~ 1.0 , nearly six times the solar value¹⁴¹. Shields and Oke⁵⁷ find a fourfold over-abundance for N/O from optical data for this object.

3C273

Like the other bright extragalactic objects discussed here the QSO 3C273 ($z_{\text{em}} = 0.158$) has been very well studied at all available wavelengths^{70-80,82,58}. Far UV observations preceding the present work were made by Wu⁸ and Davidsen *et al.*⁴². Apart from being the brightest QSO, 3C273 is also one of the most luminous. It is only moderately variable in the radio, IR, optical and X-ray regions^{54,79}. VLBI radio measurements indicate a double component apparently separating at a projected linear velocity $\sim 4c$ (ref. 80). 3C273 is among comparatively few QSOs which show the forbidden lines to be very weak, indicating that there is very little low density gas in the line-emitting region⁷⁵. It is also rare in showing strong Fe II emission^{72-75,81}.

3C273 was observed by IUE with both spectrograph cameras, as detailed in Table 1. One of the two short wavelength spectra is shown in Fig. 6. Emission features are listed in Table 5. Weak absorption features are also present, listed in Table 6, but are not easy to discern in Fig. 6 because at this time the telescope focus was rather soft (in Fig. 1 compare the raw spectrum of 3C273 with that of NGC4151 which was in sharp focus, although trailed). We identify all but one doubtful line as arising in interstellar gas in the Galaxy. Corresponding hydrogen absorption at Ly α is not observed, but this region is masked by geocoronal Ly α emission as a consequence of using the large entrance aperture.

The continuous spectrum of 3C273, with measurements averaged over bands 20–50Å wide avoiding the emission lines, is shown in Fig. 7. We include our UBV photometry, and Wu's⁸ ANS photometry, both corrected for emission lines, and the continuum data of Davidsen *et al.*⁴². Assuming the usual power law we find $\alpha \approx 1.0$ for the slope of the continuum over our whole observed UV (IUE) range, uncorrected for possible reddening. In optical range, as is well known^{42,75}, the spectrum is much flatter and contains strong Balmer continuum emission (in the U- and B-channel). Davidsen *et al.*⁴² argue that for 3C273 there is no internal reddening and for galactic reddening they deduce $E(B-V) = 0.01$; but Wu⁸ estimates $E(B-V) = 0.07$ for galactic reddening, and from an apparent absorption at redshifted λ 2200 obtains $E(B-V) = 0.13$ for internal reddening. At least for the line emitting region, internal reddening is

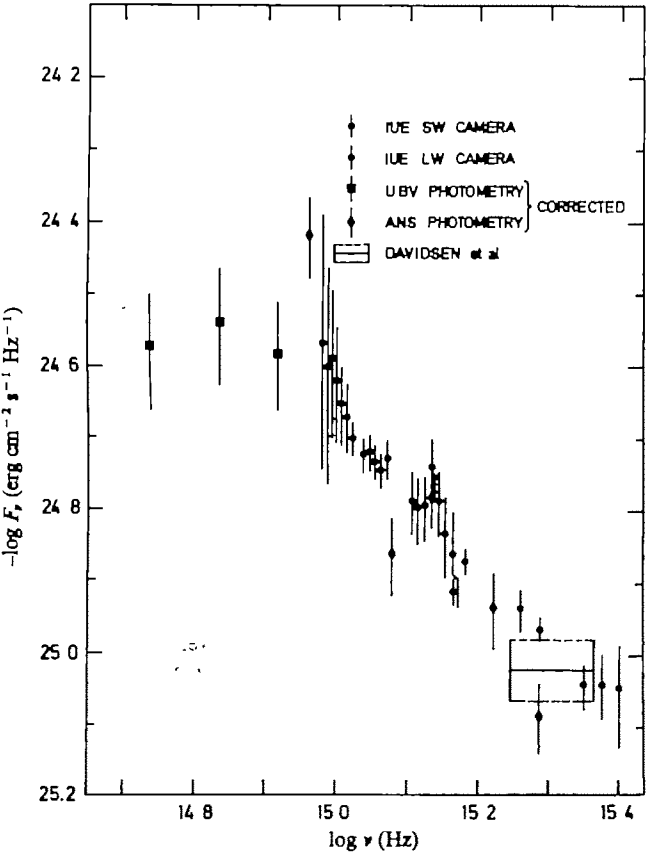


Fig. 7 3C273 continuum fluxes. The IUE data are averaged over bands 20–50 Å wide avoiding the emission lines. Our UBV photometry and the ANS photometry⁸ also are corrected for emission lines. The continuum data of Davidson *et al.*⁴² are included. No reddening correction is applied. The error bars indicate 1σ statistical uncertainties only. Although the various observations were not made concurrently, we may rely on the general character of these continuum data as 3C273 is known not to be highly variable.

suggested by the observed Balmer decrement, which is steeper than the predicted recombination values and perhaps indicates $E(B - V) = 0.69$ (ref. 75), although reddening alone does not seem a sufficient explanation⁷¹. However, reddening is not confirmed by the ratio $Pa/H\alpha$ (ref. 83), nor the helium line strengths⁷⁵, showing that the situation is more complicated than supposed^{75,73,39}. The IUE spectrum is somewhat contaminated near the redshifted position of the $\lambda 2200$ dust feature, but there is no sign at all of broad absorption at this wavelength, and on this basis we conclude that internal reddening is small for the 3C273 continuum.

3C273 shows strong line emission at $Ly\alpha$ and C IV $\lambda 1550$, moderate emission at $Ly\beta + OVI \lambda 1035$, N V $\lambda 1240$, Si IV + O IV $\lambda 1400$, C III $\lambda 1909$ and Mg II $\lambda 2800$, and has some definite or possible weaker lines, as shown in Fig. 6 and Table 5. There is no obvious difference between the UV emission line spectrum of 3C273 and those of high redshift QSOs observed optically^{52,85,86,44}, except that C III $\lambda 1909$ seems weaker than average, suggesting a relatively high density in the line emitting region. The ratio of line strengths $I(N V \lambda 1240)/I(Ly)$, ~ 0.09 , is within the range observed for high redshift QSOs, which show a wide variation in this ratio^{44,52,86,87}. Once more we find that the ratio $I(Ly\alpha)/I(H\beta)$ is much lower than predicted by recombination theory; combining our measurement of the $Ly\alpha$ flux with the value obtained by Davidson *et al.*⁴² and comparing this with previous measurements of the $H\beta$ flux^{75,84} gives $I(Ly\alpha)/I(H\beta) = 5.5 \pm 2$. Again we note the remarkable constancy of the observed ratio. Assuming no reddening in the line-emitting region but applying Wu's⁸ galactic reddening correction raises the ratio to 7 ± 3 ; using the smaller correction of Davidson *et al.*⁴² gives the value 6 ± 2 .

Comparing our value of $W_{\lambda 0}$ (C IV) for 3C273 with Baldwin's⁴³ luminosity relationship, as before, gives an expected

luminosity $\log F_{\nu}(1450) = 30.6$. Our observed value is 31.0, in good agreement. As already stated, with the available evidence the range of validity of Baldwin's relationship among active nuclei seems to be restricted to QSOs. Baldwin's result is not expected from simple photoionisation models which naturally lead to a constancy of equivalent width with luminosity, as found by Osmer⁴⁴: for a given spectral index the continuum flux is an indicator of the energy available for heating the gas and the C IV line is a good measure of the energy emitted by the gas; hence the equivalent width, that is, the line strength expressed in terms of the continuum, should be independent of luminosity. However, Osmer⁴⁴ does find a significant luminosity dependence for $W_{\lambda 0}(Ly\alpha)$, also noted by Baldwin⁴³, to an extent not expected in the photoionisation model.

The optical Fe II emission lines in 3C273 all arise from transitions between odd-parity levels at ~ 5 eV and even-parity levels at ~ 3 eV (refs 82, 113). The transitions from these 3 eV levels down to the lower-lying levels of the ground state are forbidden. These [Fe II] transitions are not observed in 3C273, indicating that the electron density $N_e > 10^7 \text{ cm}^{-3}$ in the Fe II emission region. The most likely mechanisms for producing the observed Fe II emission are resonance fluorescence of continuum radiation¹¹³ and collisional excitation^{78,82,114,115}, the former being the one generally preferred although the correlations implied by this mechanism are not observed¹⁴². The strongest Fe II lines in 3C273 belong to multiplet 42 ($\lambda\lambda 4924, 5018$ and 5169). A test of resonance fluorescence is to observe the spectral region of the resonance lines in multiplet 3UV (around $\lambda_0 2340$) and look for absorption of continuum photons¹¹⁶. No such absorption is observed for 3C273. (Fe II absorption lines are present in NGC4151, which also shows weak optical Fe II emission, but these are most likely in part due to the unusual absorbing region observed in this object and in part galactic). Model calculations^{82,113} imply nearly complete conversion of the UV continuum to explain the strength of the optical Fe II emission, so it is surprising that no obvious absorption is present. On the other hand, collisional models predict strong emission in the resonance lines⁸², which also is not observed. In fact, the fluorescence models also predict strong resonance line emission, complementing the continuum absorption in the same lines. Depending on the geometry of the line-emitting region, fluorescence models could produce either net absorption or emission in the resonance lines, so the failure to observe either does not rule out the mechanism⁸². Again, collisional models imply a very large optical depth in the resonance lines⁸² and substantially all resonance photons may then be destroyed through the leak which gives the optical lines. The effect of increasing optical depth has been discussed by Jordan¹⁴³. But then resonance fluorescence of continuum radiation would also occur, so collisional excitation alone is not likely

Table 5 Emission lines in the spectrum of 3C273				
Identification	λ_0 (Å)	$W_{\lambda 0}$ (Å)	F ($10^{-12} \text{ erg cm}^{-2} \text{ s}^{-1}$)	Comments
Lyβ	1,026			
O VI	1,035	5†	1.1†	
Si II	1,194	2†	0.3†	Probable
Si III	1,206	79	13.5	
Lyα	1,216			
N V	1,240	7	1.2	
Si II	1,263	—	—	Probable
O I	1,305	2†	0.3†	
Si IV + O IV]	1,400	5	0.8	
N IV]	1,486	4†	0.6†	Possible
C IV	1,549	58†	7.8†	Possible small contribution from Si II $\lambda 1526$
[Ne IV]	1,602	6†	0.7†	Possible
He II	1,640	4†	0.5†	Possible
C III]	1,909	10	1.2	
Mg II	2,800	17	1.6	

†Uncertain.

Table 6 Absorption lines in the spectrum of 3C273

Identification	λ_{obs} (Å)	Comments
O I	1,302	Galactic
C II	1,335	Galactic
?	1,374†	Possible
Al II	1,671	Galactic, possible

†Uncertain

to be responsible for the strong optical Fe II emission. Detailed model predictions are required to resolve this question. (See note added in proof.)

The very efficient conversion of the UV continuum into optical emission required if resonance fluorescence is the principle excitation mechanism implies 0.5–1.0 of the sky as seen from the continuum source is covered by optically-thick clouds⁸². This conclusion also seems consistent with the large equivalent width of H β generally observed^{40,82}. However, we would then expect $W_{\lambda_0}(\text{Ly}\alpha) \approx 700 \text{ Å}$ for a continuum slope $\alpha = 1^{40}$, but the observed values, as for 3C273, are about an order of magnitude smaller. The known general absence of Lyman continuum absorption in the rest frame of high redshift QSOs⁸⁶ implies that <0.1 of the continuum radiation is intercepted by optically thick clouds. This is consistent with the observed Ly α equivalent widths (if Ly α has not been strongly attenuated) and also with model calculations embodying other UV lines observed in high redshift QSOs⁵², yet is inconsistent with the H β (and Fe II) strengths which become anomalously high for a covering factor <0.1 .

Narrow absorption lines resembling the interstellar lines of our Galaxy are frequently observed in the spectra of QSOs and BL Lac objects. In general, these lines may be grouped into one or more systems corresponding with the apparent wavelengths of transitions in a physically plausible group of atoms or ions exhibiting the same, although arbitrary, redshift^{88,89}. Such absorption systems range in redshift from being comparable with the associated emission line redshift, to having very much smaller values which in some cases give a velocity relative to the QSO exceeding 0.6c. The general character of the absorption lines does not seem to differ for systems widely ranging in redshift, and velocity relative to the QSO. However, for QSOs of sufficient redshift to bring the Ly α emission line into the observable optical range, it is striking that most of the absorption lines fall shortwards of Ly α emission, and that the bulk of these cannot be grouped into convincing absorption systems. It is generally accepted that these unidentified lines represent Ly α absorption in gas extending to lower redshifts than the QSO against which they are observed and for which lines of other elements are too weak to be detected⁹⁰. In support of this is the frequent identification in these cases of one or more other members of the Lyman series^{91,92}.

The question of the origin of QSO absorption lines has been greatly discussed^{7,93–106}. In principle, the narrow-line absorbing regions may be intrinsic to the QSOs, having been somehow ejected at velocities up to the very high values observed, or they may be cosmologically distributed in direct association with galaxies or as intergalactic clouds. The outflow hypothesis has several difficulties, principally the implication of implausibly large mass and momentum flow relative to the QSO^{97–100}, and the balance of evidence now strongly favours the cosmological hypothesis. On the other hand, the very broad-line absorption present in some QSOs is probably intrinsic⁷.

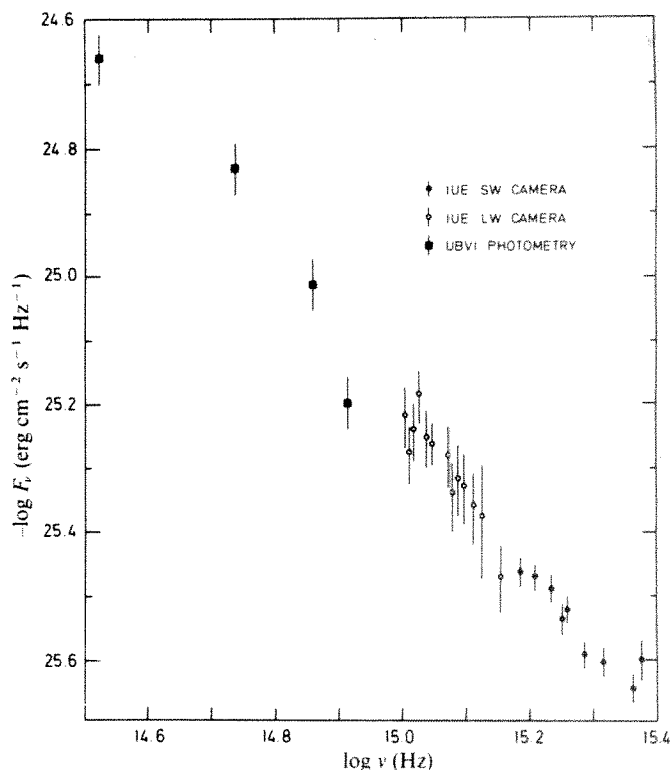
From the observed frequency of absorption systems containing heavy elements, if related to galaxies, the effective size of a galaxy seen in absorption must be several times its arbitrary Holmberg dimensions^{7,94,106,107}. However, as pointed out by Bahcall and Spitzer¹⁰⁸ the gas density required to produce absorption lines is so low that it would not be surprising if the maximum dimensions for measurable absorption in a line were much greater than the dimensions detected in other ways, and they suggested that the observed lines could be produced in

extended low density haloes of normal galaxies. Direct evidence that heavy element absorption lines in QSO spectra can be caused by the interception of the outer regions of spiral galaxies well outside the Holmberg dimensions comes from the detection by Boksenberg and Sargent¹⁰⁹ of Ca II absorption lines in the spectrum of the QSO 3C232 ($z_{\text{em}} = 0.5303$) at the same redshift as the galaxy NGC3067 which lies 1.9 arc min away in the plane of the sky, or about 1.5 Holmberg radii (using $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$), following the discovery by Haschick and Burke¹¹⁰ of H21 cm absorption in this system. Taking into account the inclination of NGC3067, the line of sight intersects the plane of the galaxy at a radial distance greater than 6 Holmberg radii out from the centre. However, the characteristics of the absorption system point to it arising in the outer halo of NGC3067 not the disk extension¹⁰⁹.

For 3C273 we find absorption lines only at zero redshift (Table 6), presumably arising in our Galaxy. Corresponding galactic Ca II absorption has been reported by Greenstein¹¹¹. Wagoner¹¹² has computed the probability that light received from a distant QSO be intercepted by an intervening galaxy and show absorption lines. Assuming an effective cross-section 10 times the area defined by the Holmberg dimensions, Wagoner's data still give only 0.05 as the probability of interception along the line of sight to 3C273, which is consistent with our null detection. This further supports the cosmological hypothesis for the absorbing material. On the outflow hypothesis the probability of detecting absorption systems is not expected to depend strongly on the redshift of the QSO.

Turning to the consideration of the Ly α absorption lines normally seen optically in the spectra of high redshift QSOs, even if the resolution of our 3C273 spectrum were not sufficient to separate these lines, if present, we would expect them to blend into a marked depression of the apparent continuum shortwards of Ly α emission. Neither we nor Davidsson *et al.*⁴² observe such a depression. The observed number density of the Ly α absorption lines seems to increase quite rapidly with redshift, with objects of $z_{\text{em}} \sim 2$ showing a markedly lower absorption line density than those of $z_{\text{em}} \sim 3$. Our null detection for 3C273 follows this trend and again supports the cosmological hypothesis. There is

Fig. 8 B2 1101+38 continuum fluxes. The IUE data are averaged over 50 Å bands. The UBVI photometry are approximately concurrent with the IUE measurements (see Table 1). Errors are estimated only from the 1 σ statistical uncertainty in signal. No reddening correction is applied.



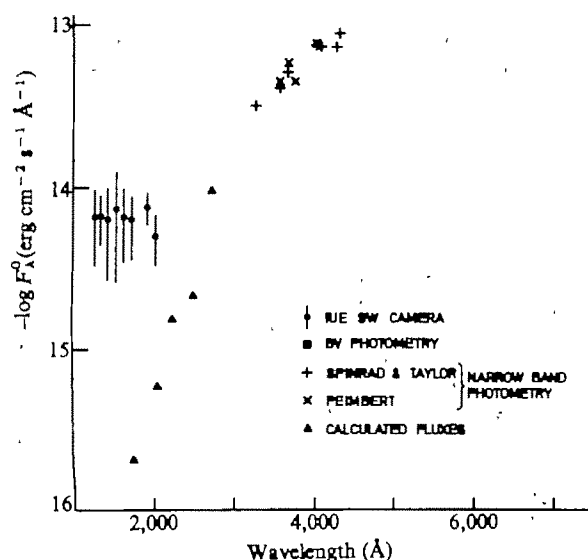


Fig. 9 Spectrophotometry and photometry of the central region of the spiral galaxy M81. The IUE short wavelength measurements are averaged over 100 Å bands. The errors define 1σ statistical uncertainties in signal only. The BV photometry (from ref. 127) and the narrow band photometry are reduced to the IUE observing aperture. The data are de-reddened using $E(B-V) = 0.06$. The derivation of the calculated fluxes is described in the text.

evidence that the Lyα absorption systems represent in part very extended outer regions of galaxies where the density is too low to allow star formation and the gas still has primordial composition, and in part truly intergalactic gas not bound to galaxies⁷.

B2 1101+38

B2 1101+38 (= Markarian 421) is a radio-faint galaxy¹¹⁷ identified as a BL Lac object^{118,119} and also is an X-ray source^{120,144}. It is among a few BL Lac objects observed to be surrounded by a nebulosity. Three absorption features in the spectrum of the nebulosity are consistent with being Ca II K, H and the G-band at $z = 0.03$ (refs 118, 121) and may be interpreted as originating from the main body of an elliptical galaxy having an extremely bright nucleus. BL Lacertae itself is similar, with $z = 0.07$ (refs 122–125). Five galaxies within 15' of B2 1101+38 are found also to have $z = 0.03$, showing that this object belongs to a cluster of galaxies¹²¹.

BL Lacertae objects in general appear similar to variable QSOs except that they lack strong optical emission lines. For a review of BL Lac objects see Stein *et al.*¹²⁶. Attempts to explain the absence of emission lines are usually based on (1) the supposition of a lack of UV photons to ionise a surrounding gas; (2) the absence of any such gas; or (3) a combination of both.

In our extension into the far UV there are still no emission-lines evident in the spectrum of B2 1101+38. There is, however, a strong non-thermal continuum. In Fig. 8 we show the IUE continuum measurements and include our UBVI photometry (see Table 1). Assuming a power law spectrum of the usual form $F_\nu \propto \nu^{-\alpha}$ we obtain $\alpha = 1.2$ as a mean value over the observed range. The radio spectrum is flatter, with $\alpha = 0.26$. As there is no sign of Lyα absorption at $z = 0.03$ (which would be clear of the geocoronal Lyα emission) we may assume that the observed UV spectrum extends well into the Lyman continuum. This is supported by the measurements of the possible X-ray counterpart by Ricketts *et al.*¹²⁰ which indicate a steady component of 0.7 Ariel 5 count s⁻¹ (sometimes brightening by up to a factor 20) while extrapolation of our continuum predicts 0.5 counts s⁻¹. There may be slight reddening, as our data are too noisy to confirm the absence of a weak absorption feature at local or redshifted λ 2200, but there is no strong reddening so indicated.

If photoionisation is the source of line emission we may calculate the equivalent width of Hβ in terms of the photoionising continuum, assuming that the gas which gives rise to line emission is optically thick and completely surrounds the

continuum source. Using the expression given by Penston and Fosbury⁸⁴ (taking a continuum high-frequency cutoff at λ 228) we obtain $W_\lambda(H\beta) \approx 50 \text{ Å}$ for $\alpha = 1.20$. (There is an omission in their expression as given; the correct expression is

$$W_\lambda(H\beta) \approx 564 \left(9.64 \frac{0.553}{K} \right)^{-\alpha} \left[\frac{K^{-\alpha} - 1}{\alpha} \right]$$

where $K = 0.553$ or 0.250 with the continuum high-frequency cutoff at λ 504 or λ 228 respectively, and α is defined above). If the intensity ratio of Lyα to Hβ is ≈ 3 , as is now commonly observed or deduced (see above) and using

$$I(\text{Ly}\alpha)/I(H\beta) = (4861/1216)^{-\alpha} W_\lambda(\text{Ly}\alpha)/W_\lambda(H\beta),$$

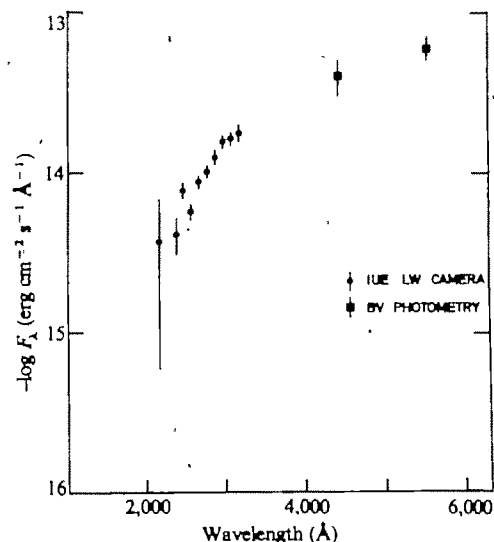
then $W_\lambda(\text{Ly}\alpha) \approx 50 \text{ Å}$. Our observed upper limit is $W_\lambda(\text{Ly}\alpha) \leq 10 \text{ Å}$. In the previous section we have discussed the conflicting evidence regarding complete (or nearly complete) coverage of the photoionising source. However, the Hβ strength normally observed in QSOs and Seyfert galaxies is well explained in this way. Thus we may conclude at least for B2 1101+38, and probably for BL Lac objects in general, that the absence of emission lines is due not to a deficiency of ionising flux, but of gas in the physical or geometrical state normally found in the line-emitting regions of low redshift QSOs. A similar conclusion follows from the lack of detectable X-ray absorption in such objects¹⁴⁴.

From Baldwin's⁴³ empirical relationship coupling $W_{\lambda_0}(\text{C IV})$ with $L_\nu(1450)$, discussed above for NGC4151, NGC1068 and 3C273, we expect from our continuum flux that $W_{\lambda_0}(\text{C IV}) = 590 \text{ Å}$, but our observed upper limit is $W_{\lambda_0}(\text{C IV}) \leq 15 \text{ Å}$. Again we have a serious departure from Baldwin's empirical relation; it is interesting that B2 1101+38 has a flat radio spectrum and Baldwin finds his correlation is particularly tight for QSOs with such spectra.

M81

The central region of the well-studied spiral galaxy M81 was observed only with the short wavelength spectrograph on IUE (Table 1). The signal obtained was low, and to increase the signal-to-noise ratio we binned the data into 100 Å bands, as shown in Fig. 9 which also shows the BV photometry of Brandt *et al.*¹²⁷ and the narrow band photometry of Spinrad and Taylor¹²⁸ and Peimbert¹²⁹. The Spinrad and Taylor results were normalised to Peimbert's at λ 5360. These are all reduced effectively to the same observing aperture as used for the IUE data. All the observed data are de-reddened using $E(B-V) = 0.06$ (ref. 128), and the extinction law of Code *et al.*³⁸ in the UV.

Fig. 10 Spectrophotometry and photometry of the central region of the giant elliptical galaxy M87. The long wavelength IUE data are averaged over 100 Å bands and include only 1σ statistical uncertainties in signal. The photometry are from refs 137 and 139 and are reduced to the IUE observing aperture. No reddening correction is applied.



We also show a theoretical extension of the optical data into the UV calculated from the stellar population of Spinrad and Taylor¹²² and the appropriate UV stellar fluxes from the TD1 S2/68 instrument¹⁰.

The observed IUE spectrum clearly does not arise from the assumed stellar population, whose emission is dominantly from G-type subgiants, but in magnitude agrees well with Peimbert's¹²⁹ estimate of the fluxes required from a hot star population (late O-type) needed to explain the intensity of the observed H α and [O III] emission in this object. However, our flux distribution has the wrong slope for Peimbert's assumed stellar population, and is fitted better by a mean A0 spectrum, but this will not be able to provide the required photoionising flux. Alternatively, the observations are well-explained by a non-thermal continuum source of spectral slope $\alpha = 2$. The radio nucleus of M81 is known to be variable and is about 10^4 times more luminous than the compact radio source at the galactic centre⁸⁰.

M87

The peculiar EO galaxy M87 (=NGC4486=Arp 152=3C274=Virgo A) is an active (core-halo) radio source with compact central components^{130,131}. It also has compact and extended X-ray emission¹³⁴⁻¹³⁶, and there is evidence for ejection of mass from the nucleus in the form of optical jets^{132,133}, and for a massive black hole in the nucleus^{137,138}.

M87 was observed by IUE only with the long wavelength spectrograph. Again we binned the data into 100Å bands to increase the signal-to-noise ratio. The results are shown in Fig. 10. We include BV photometry from de Vaucouleurs and Nieto¹³⁹ and Young *et al.*¹³⁷, reduced to the same observing aperture as for the IUE data.

From our UV data, an early G-type spectrum is indicated for the nuclear region of M87. Late G-type to early K-type (dominant in the visible flux) is too cool, as for this an absorption edge cuts off the flux below $\sim \lambda 2640$. F-type subgiants can be ruled out as the slope is wrong and the absorption dip at $\lambda 2400$ present in F-type stars¹⁰ is not evident in the IUE spectrum. Actually, there is marginal evidence for an emission peak (perhaps [Ne IV] $\lambda 2424$). There may also be some contribution from a non-thermal continuum, which is expected for this object.

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Note added in proof. Recent computations^{145,146} suggest observational evidence does point strongly to collisional excitation as the dominant mechanism producing Fe II optical emission lines

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IUE observations of Solar System objects

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During the scientific commissioning phase of IUE several spectra were acquired from objects residing in the Solar System. The activities focused on testing numerous parameters which would indicate the usefulness of IUE for planetary science. It seems that IUE can successfully tackle many important questions and the data analysis and interpretation of the initial set of observations has begun.

IUE PROVIDES an excellent way for acquiring UV spectra in the wavelength region shortwards of the Earth's ozone cutoff (about 3,100 Å). The UV vidicons, when properly erased and prepared for use, can be exposed for several hours on very weak planetary emissions. This integration time, and the ability of the spacecraft to be adjusted to track on planets or their natural satellites for extended periods, combine to provide an overall capability which is well beyond the reach of sounding rockets. The low resolution channel (linear spectrum) has a spectral resolution of 7–8 Å FWHM, much like the classical rocket and satellite spectrometers now in use. The high resolution mode (echelle spectrum) gives a spectral resolution in the range 0.1–0.2 Å FWHM. (The scientific instrument is described by Boggess *et al.*¹) Either the large 10 × 20 arc s slot or the 3 arc s circular aperture can be used for studies requiring spatial resolution, if the tracking can be adjusted and maintained to ~ 1 arc s during an exposure. The sample spectra which were recorded during the IUE commissioning phase show some of the possibilities for future studies with Solar System objects. Table 1 represents the wide range of objects examined. The efforts centred around acquiring exposure times for reasonable signal-to-noise ratio spectra and becoming familiar with the IUE capability. No definitive study of any one object was performed, but an overall analysis of what further studies would be possible was accomplished.

Figure 1 reproduces a single long-wavelength spectrum of Mars, acquired through the small, 3 arc s aperture. Useable information is obtained down to 2,200 Å. The decrease in signal strength longwards of 2,900 Å is the result of the threshold of

the caesium telluride photocathode in the UV quantum converter which is in front of the vidicon faceplate. The spectrum was obtained by placing the small aperture over the 8 arc s diameter disk of Mars, and using the fine error sensor (FES) to lock onto the 'spill-over' light. The exposure was short enough (15 s) to not require trimming of the spacecraft gyros to the non-sidereal motion of Mars. The strong Mg I and Mg II solar absorption lines are easily detected at 2,852 and 2,795 and 2,802 Å, respectively. Just longwards of 2,900 Å, the spectrum saturated slightly, but the data became valid again at 2,960 Å. Although the signal is not strong in the 2,400–2,600 Å region, no pronounced absorption band is seen around 2,550 Å where the Hartley band system of ozone has its maximum absorption. If substantial ozone were present in the martian atmosphere, one would expect to see an absorption bowl in the Mars spectrum². To enhance the detectability, within the limits of the statistics of a single spectrum, the Mars spectrum was compared with a lunar spectrum also obtained with IUE. This is displayed in Fig. 2. No apparent minimum is observed and the slope of the ratio represents the albedo differences of the two bodies. The Mars spectrum was recorded while its northern hemisphere was in late spring ($L_s = 70^\circ$) and the north polar region was tipped towards Earth, substantially obscuring the south polar region. In

Table 1 Objects from the Solar System observed using IUE

Object	Wavelength range	Dispersion
Mars	Long	High and low
	Short	Low
Jupiter	Long	High and low
	Short	Low
Saturn	Long	Low
Uranus	Long	Low
	Short	Low
Io	Long	Low
Ganymede	Long	Low
Titan	Long	Low
Moon	Long	High and low
	Short	Low

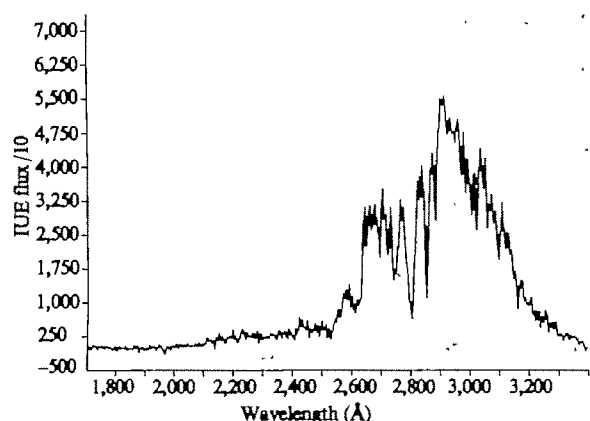


Fig. 1 Long wavelength, low resolution spectrum of Mars recorded on April 1978 with a small aperture. The system response function has not been removed. Exposure time is 15 s.

this configuration the northern hemisphere contributed ~60–65% of the total reflected light. The water vapour content of the Mars lower atmosphere becomes considerable during the late spring and its subsequent photodissociation leads to a process which inhibits ozone formation^{3–7}. Mariner 9 observed about 10 μ atm of ozone near the north pole of Mars at the equivalent seasonal time in 1972 (ref. 6), but detected no ozone near the equatorial region. Because the aperture was locked onto the centroid of light at least $1\frac{1}{2}$ arc s of the north polar region of Mars was excluded from the spectrometer. It is highly unlikely that ozone would have extended southwards from the north polar region to latitudes as far south as $+45^\circ$. Thus, if the seasonal characteristics in 1978 were similar to those in 1972, virtually no ozone should have been in the martian atmosphere which was included in the aperture. Confirming studies of the ozone seasonal characteristics will have to wait until about December 1978 when the winter–spring transition can be observed in the southern hemisphere and when one should expect to detect atmospheric ozone.

Acquiring data from the jovian system requires special considerations and techniques. Figure 3 displays the reflected light spectrum of Io (J1), Jupiter's innermost galilean satellite. The region near 2,900 Å is slightly saturated and fiducial marks on the camera faceplate account for the two apparent absorption features near 2,580 and 3,305 Å. An observation time was chosen when Io and Ganymede were simultaneously near elongation, as seen from Earth, and had minimal differential motions for several hours. The tracking procedure required FES acquisition of Jupiter, which is much too large and too bright to be useable for the tracker, followed by FES lock-up on Ganymede. The spacecraft gyros were trimmed to the Ganymede celestial motion and Io was subsequently manoeuvred into the large slot. The FES continued to lock on Ganymede while the 10 min exposure was in progress. Direct tracking on Io with the 'spill-over' light surrounding the large or small aperture (the

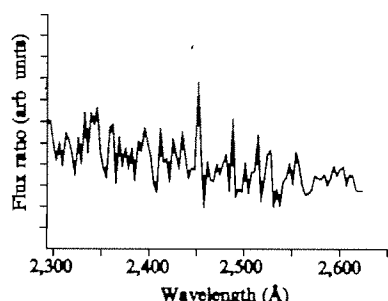


Fig. 2 Ratio of the spectrum of Mars (Fig. 1) to that of the Moon. Both spectra were recorded with IUE and the ratio was constructed from data which had received identical processing. The flux ratio is in arbitrary, linear units. No ozone absorption band is observed at 2,550 Å.

method used for Mars) is generally not possible because the scattered light from Jupiter is about equal to the 'spill-over' light from Io at Io's position in the tracker field. Europa (J2) may also have to be handled by this differential guiding technique, but Ganymede and Callisto are sufficiently removed from the visible scattered light domain of Jupiter to be directly tracked most of the time. Whenever any satellite is too close to Jupiter, within ~7 jovian radii, differential tracking will be necessary. This condition will also be true for satellite studies of the saturnian system. The spectrum of Io has not undergone extensive analysis because the data were acquired during a high radiation background portion of the IUE orbit and more background noise removal is necessary before proceeding further. Nonetheless, acquisition of Io's spectrum certainly demonstrates one of the capabilities of IUE.

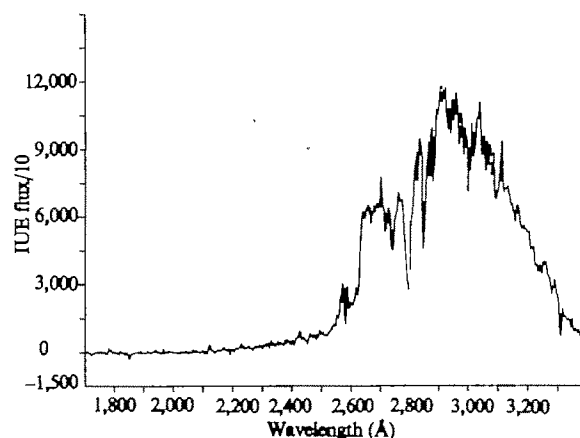


Fig. 3 Long wavelength, low resolution spectrum of Io using a large slot. The system response function has not been removed. Exposure time is 10 min.

The observation of Solar System objects with IUE is more complicated than that required for the classes of stars and galaxies for which IUE was originally designed. The significant differences are: (1) the range of visual magnitude brightness is very large, from -4 for Venus to fainter than +11 for some satellites of Saturn. This complicates acquisition and tracking. (2) Most of the bodies in the Solar System move at non-sidereal rates. Special tracking procedures need to be developed to acquire spectra of planetary satellites, regions of the bright disks of Jupiter, Saturn, Mars, Venus and the Moon. (3) All of the objects in this class echo the time variable solar spectrum, to first order. To examine the spectral range from 1,150 to 3,200 Å requires numerous exposures to cover a 10^5 brightness range with a detector system which is linear over about a ratio of 25 in object brightness in the UV. (4) Perhaps the most pressing complication facing IUE planetary observers is the acquisition of comparison solar spectra. Ideally they should be acquired by IUE itself so that all of the instrumental parameters are removed. IUE cannot look directly at the Sun, but the fully illuminated disk of the Moon near zero phase angle seems to be the best current choice for monitoring the solar spectrum. Some minor tracking difficulties still remain for accomplishing this, but they seem to be solvable. Alternate G type stars are being examined as another possibility to tie together solar spectral references.

All of these difficulties are temporary and excellent progress is being made to develop a powerful, useable system for the study of the Solar System in the UV. A brief inspection of the data acquired so far confirms that IUE is capable of providing measurements which can supply crucial answers to several of the more significant problems in planetary science.

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articles

Nucleosomes are assembled by an acidic protein which binds histones and transfers them to DNA

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The nucleosome subunits of chromatin are assembled from histones and DNA by an acidic protein which binds histones. The nucleosome assembly protein has been identified and purified from eggs of Xenopus laevis.

DNA in eukaryotic chromosomes is packed into a repeating chain of nucleosome subunits, each consisting of an octamer of histones complexed to approximately 200 base pairs of DNA (for reviews see refs 1 and 2). This article describes the identification and purification of a protein which binds histones and transfers them to DNA to form nucleosomes. The nucleosome assembly protein has been purified from unfertilised eggs of the frog *Xenopus laevis* by fractionating a cell-free nucleosome assembly system³. The cell-free system converts purified DNA into a regularly repeating chain of nucleosomes at a rate of 5×10^7 nucleosomes per s per egg³ using either the egg's endogenous histone pool⁴⁻⁶ or exogenous sources of purified histones. Assembly is not dependent on DNA synthesis³. The products obtained when DNA is incubated in the cell-free system were recognised as chains of nucleosomes by the criteria of: (1) sedimentation, (2) buoyant density, (3) beaded appearance in the electron microscope, (4) localised resistance to micrococcal nuclease, (5) histone dependence and (6) insertion of superhelical turns into relaxed circular DNA^{3,7}. We have used histone dependent insertion of superhelical turns into relaxed circular DNA as a rapid assay for nucleosome assembly because Germond *et al.*⁸ have shown that the number of superhelical turns is directly related to the number of nucleosomes on the DNA. This supercoiling activity should not be confused with the DNA gyrase of prokaryotes which also inserts superhelical turns into DNA⁹. In contrast to the activity we describe here, DNA gyrase requires ATP but does not require stoichiometric amounts of structural proteins such as histones, and in addition its activity is counteracted by nicking closing enzyme⁹.

An assembly factor is required for ordered interaction of histones with DNA

Nucleosomes can be reconstituted from separated DNA and histones by prolonged dialysis from $>1\text{M NaCl} \pm 5\text{M urea}$ (reviewed in ref. 2), but at physiological ionic strength mixtures of DNA and histones precipitate. Addition of small amounts of *Xenopus* egg homogenate prevents precipitation and results in nucleosome formation^{3,7}, suggesting that eggs contain an additional factor which is required for the ordered interaction of histones with DNA to form nucleosomes at physiological ionic strength.

The nucleosome assembly factor is a protein

To determine the nature of the assembly factor in eggs of *Xenopus laevis*, egg homogenates were incubated with a variety of specific degradative enzymes before assaying for assembly activity. The results are summarised in Table 1. The assembly factor is unlikely to contain a nucleic acid component since it is totally resistant to ribonuclease A, and micrococcal nuclease.

The assembly factor is also resistant to trypsin and chymotrypsin, but assembly is only observed if the proteases are inhibited by phenylmethylsulphonyl fluoride (PMSF) after digestion and if exogenous histones are added. (Exogenous nicking-closing enzyme is also required after digestion in order to visualise assembly by monitoring insertion of superhelical

Table 1 Sensitivity of the assembly factor to degradative enzymes

Enzyme preincubated with egg supernatant	Enzyme inhibitor	Assembly activity (using added histones)
Ribonuclease A ($500\text{ }\mu\text{g ml}^{-1}$)	Nil	+
Micrococcal nuclease ($1,500\text{ units ml}^{-1}$) + 2 mM CaCl_2	EDTA (5mM)	+
Trypsin ($250\text{ }\mu\text{g ml}^{-1}$)	PMSF†	+
Chymotrypsin ($<1\text{ mg ml}^{-1}$)	PMSF†	+
Proteinase K ($250\text{ }\mu\text{g ml}^{-1}$)	PMSF†	—
PMSF + proteinase K ($250\text{ }\mu\text{g ml}^{-1}$)	Inhibited before incubation	+

Xenopus egg homogenates were prepared and clarified as described previously³ except that 0.66 volumes of 120 mM KCl , 2 mM MgCl_2 , $20\text{ mM Tris-HCl pH } 7.5$ were added after homogenisation and the $1,800\text{ g}$ supernatant was centrifuged twice at $145,000\text{ g (max)}$ for 30 min. $20\text{ }\mu\text{l}$ of clarified homogenate was incubated with each enzyme for 1 h at 25°C . Enzymes were then inhibited before addition of $1\text{ }\mu\text{g calf thymus histones}$, $0.5\text{ }\mu\text{g SV40 form I relaxed DNA}$ and nicking-closing enzyme which had been partially purified from eggs of *X. laevis* by chromatography on DEAE-cellulose and phosphocellulose columns¹². Assembly was assayed by incubation at 20°C for 3 h followed by agarose gel electrophoresis to reveal superhelical turns¹³. PMSF was dissolved in ethanol at 6 mg ml^{-1} and added to homogenates to give a final concentration of $150\text{ }\mu\text{g ml}^{-1}$.

* When micrococcal nuclease was not inhibited following pre-incubation, the SV40 DNA was completely degraded.

† When PMSF was omitted ^{35}S -labelled histones added to the incubation were digested to complete solubility in 5% trichloroacetic acid containing 0.25% sodium tungstate.

turns into relaxed DNA.) The resistance to trypsin and chymotrypsin suggests either that the assembly factor is not a protein or that it is a protein which lacks exposed basic and aromatic amino acids on its surface. To distinguish these two possibilities we tested its sensitivity to proteinase K (BDH, twice crystallised), a single protease which cleaves most types of accessible peptide bond and which is completely and irreversibly inhibited by PMSF¹⁰. The assembly activity is abolished by incubation with proteinase K. The destruction of activity is more rapid and more complete when the preincubation is performed above pH 8. Addition of histones with or without nicking-closing enzyme fails to restore any activity. When the proteolytic activity of proteinase K preparations is inactivated by PMSF before incubation with egg homogenates the assembly activity remains undiminished (Table 1), confirming that the loss of activity caused by proteinase K is due to proteolysis.

Identification and purification of a protein which co-purifies with assembly activity

To identify any proteins which co-purify with the assembly activity, clarified egg homogenates (see Table 1 legend) were initially fractionated by sedimentation through a 10–40% sucrose gradient. The distribution of polypeptides in the gradient fractions was examined by polyacrylamide gel electrophoresis of aliquots (Fig. 1a). Simultaneously each fraction was assayed for its ability to insert superhelical turns into SV40 circular DNA which had been relaxed by nicking-closing enzyme as described previously³. The number of superhelical turns is related to the number of nucleosomes on the DNA⁸. Figure 1b demonstrates that a sharp peak of activity is observed. The sedimentation rate of the most active fraction was previously estimated as "approximately 11S"⁷. Centrifugation for longer times in a rotor which generates greater force (SW 60Ti) collection and assay of a larger number of fractions and comparison with a wider range of markers (catalase, glyceraldehyde-3-phosphate dehydrogenase and alkaline phosphatase) reveals that this is an overestimate and that the value is nearer to 8S. Figure 1a shows that the active fractions contain a wide range of polypeptides, but that many others are removed by this simple fractionation.

When crude undiluted homogenates are heated to 80 °C for 10 min a gelatinous precipitate forms and assembly activity is lost, hence we described the activity as thermolabile³. However, when the active fractions from a sucrose gradient (or diluted, twice reclarified homogenates) are heated 10 min at 80 °C the supercoiling activity is not precipitated; it is simply made dependent on added histones and nicking-closing enzyme. Figure 1c shows that heating 10 min at 80 °C followed by cooling and centrifuging 2 min at 10,000g to remove precipitated proteins causes a dramatic simplification of the polypeptide profile. One major polypeptide (arrow in Fig. 1c) remains. It has an apparent molecular weight of 29,000, judged by comparison with known standards on SDS-gel electrophoresis (superoxide dismutase, 20,000; aldolase, 30,000; glyceraldehyde-3-phosphate dehydrogenase, 36,000; creatine kinase, 40,000; ovalbumin, 43,000). It migrates slightly slower than histone H1 (which migrates as though it has an apparent molecular weight of 27,500 in the electrophoresis conditions used here). The polypeptide of MW 29,000 is not visible in the unheated sample (Fig. 1a). It is obscured by a more prominent band which migrates just ahead of it (which is not histone H1). Note that the distribution of the polypeptide marked by an arrow in Fig. 1c is similar to the distribution of assembly activity in Fig. 1b. Most of the minor bands in the fraction marked by an arrow can be removed from the heated gradient fraction by a second centrifugation at 10,000g for 2 min. To obtain optimal precipitation of other proteins it is necessary to add 0.025 volumes of 6 mg ml⁻¹ PMSF in ethanol before heating. Ethanol alone substitutes partially but not completely.

Figure 2 shows that assembly activity also co-purifies with a polypeptide which has an apparent molecular weight of 29,000

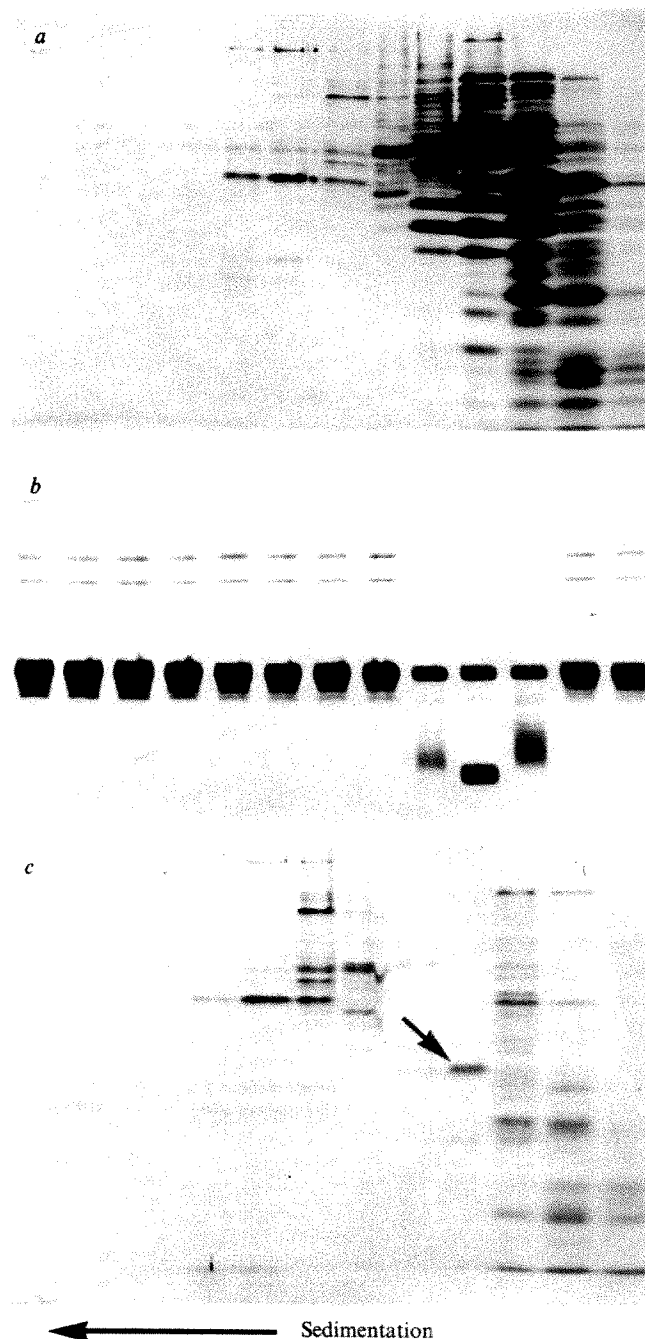


Fig. 1 Nucleosome assembly activity co-sediments with a particular protein in a sucrose gradient. *a*, SDS gel of proteins; *b*, DNA supercoiling assay; *c*, SDS gel of proteins as (*a*) which resist heat precipitation. Details are as follows: *a*, Separation of polypeptides contained in each sucrose gradient fraction by electrophoresis in a 15% polyacrylamide gel as described previously¹⁴, and visualised by Coomassie blue staining. *b*, Assay of each gradient fraction for insertion of superhelical turns into relaxed circular ³H-SV40 DNA. DNA configurations are resolved by electrophoresis through a 1% agarose gel and visualised by fluorography using 3% PPO in ethanol⁵. 0.1 µg of ³H-SV40 relaxed DNA was incubated in 50 µl of each gradient fraction for 3 h at 20 °C. *c*, Same as (*a*) but gradient fractions were heated 10 min at 80 °C (after addition of 0.025 volumes 6 mg ml⁻¹ PMSF in ethanol), cooled at 0 °C for 5 min, centrifuged 2 min 10,000g, at 4 °C to remove precipitated proteins and only the supernatant was treated with SDS for electrophoresis. Before gradient centrifugation 500 µl clarified egg homogenate was extracted twice with an equal volume of 1,1,2-trichlorotrifluoroethane to remove lipids and lipoproteins (such as yolk), then diluted with 100 µl of 20 ml tris pH 7.5, 5 mM EDTA. It was centrifuged through a 10–40% sucrose gradient⁷ for 16.5 h at 38,000 r.p.m. in a Beckman SW 40Ti rotor at 4 °C. Aliquots of 50 µl were prepared for electrophoresis (*a* and *c*) and assayed for assembly (*b*).

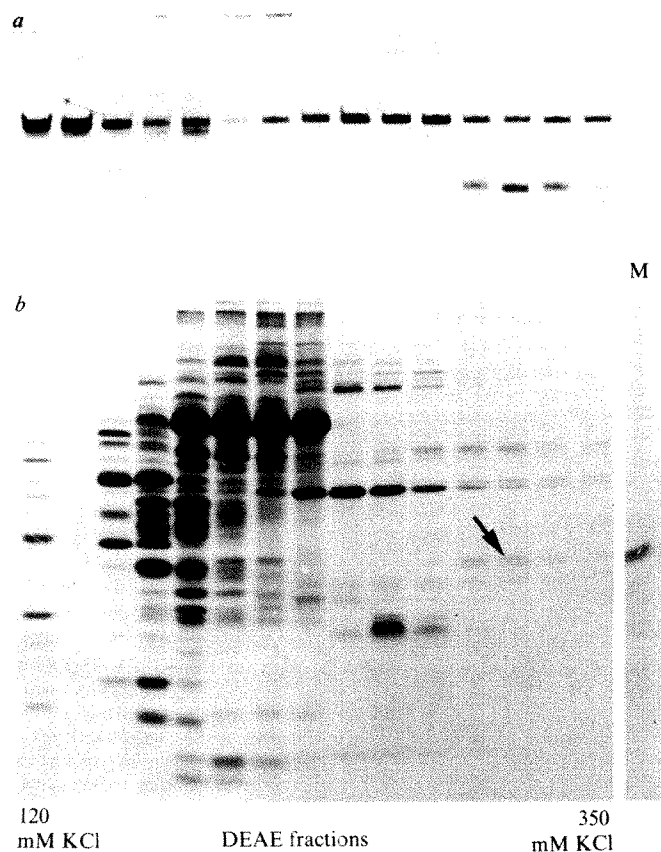


Fig. 2 Nucleosome assembly activity co-elutes with a polypeptide of MW 29,000 during anion exchange chromatography on DEAE-cellulose. *a*, Assay of each DEAE-cellulose column fraction (15 μ l diluted to 50 μ l at final KCl concentration of 120 mM) for insertion of superhelical turns into relaxed SV40 circular DNA (0.25 μ g) in the presence of calf thymus histones (0.5 μ g) and nicking-closing enzyme (see Table 1 legend). Products of a 3 h incubation at 20 °C were analysed by agarose gel electrophoresis and ethidium bromide staining. A negative image is shown to facilitate half-tone reproduction. *b*, Electrophoretic analysis of polypeptides contained in each column fraction on a 15% polyacrylamide SDS gel in conditions of Fig. 1*a* and *c*. The marker track M is the material from the track marked with an arrow in Fig. 1*c*, re-electrophoresed on this gel (that is, a heated sucrose gradient fraction). 6 ml of clarified egg homogenate was chromatographed on a 9 $\times$ 3 cm DEAE-cellulose (Whatman DE 52) column and eluted with a 150 ml linear KCl gradient from 0.12 M to 0.5 M KCl in 20 mM Tris-HCl pH 7.5. 12- μ l aliquots were diluted to 52 μ l at a final concentration of 120 mM NaCl for assay. Fractions eluting after 350 mM KCl are not shown. They contained very few proteins and showed no activity.

when clarified homogenates are fractionated by charge. Thus both the polypeptide and the activity bind tightly to the anion exchanger DEAE-cellulose suggesting that the assembly factor is highly negatively charged. After chromatography on DEAE-cellulose the assembly factor requires the addition of histones and nicking-closing enzyme in order to insert superhelical turns into relaxed DNA. When the protein of MW 29,000 is purified by heating sucrose gradient fractions and then chromatographed on DEAE-cellulose it again co-elutes with the assembly activity.

Therefore the activity which inserts superhelical turns into relaxed DNA co-purifies by both size and charge with a protein whose subunits have an apparent MW of 29,000 on SDS gels.

Figure 3*a* demonstrates the electrophoretic purity of the polypeptide prepared by sucrose gradient centrifugation followed first by heating for 10 min at 80 °C as in Fig. 1*c* and then by chromatography on DEAE-cellulose (as in Fig. 2).

Figure 3*b-d* shows that the preparation containing the purified protein illustrated in Fig. 3*a* causes the insertion of superhelical turns into relaxed DNA in the presence of acid extracted histones and nicking-closing enzyme. In the absence of the material shown in Fig. 3*a* or of the histones, assembly does not occur (Fig. 3*b-d*). Most nucleic acid contaminants are removed from the polypeptide of MW 29,000 by DEAE chromatography, but to ensure that nucleic acids or oligonucleotides are not required for its activity, a separate preparation of the protein (prepared without the final DEAE step) was incubated with 100 μ g ml⁻¹ ribonuclease A and 150 units ml⁻¹ micrococcal nuclease (in 2 mM CaCl₂) for 2 h at 25 °C followed by chromatography on Sephadex G-75 to remove oligonucleotides, CaCl₂ and the nucleases. Figure 3*e-g* illustrates that assembly activity is not decreased by this procedure.

All preparations which have demonstrated assembly activity have also contained the polypeptide of 29,000 MW. The sedimentation and gel filtration properties of the native form of this polypeptide suggest that the native protein is unlikely to consist of less than four subunits of MW 29,000.

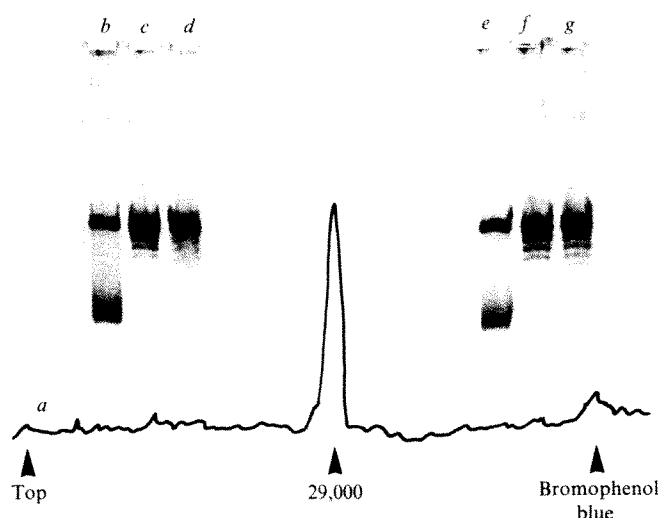


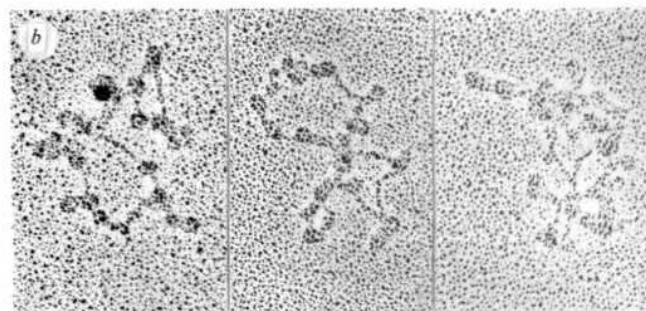
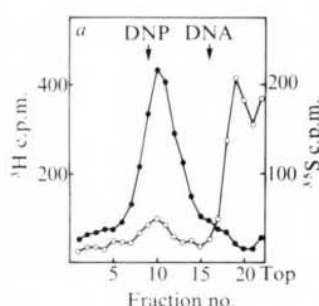
Fig. 3 The purified protein of 29,000-dalton subunits inserts superhelical turns into relaxed DNA in the presence of histones and nicking-closing enzyme. *a*, Electrophoretic purity of the polypeptide of MW 29,000 prepared by sucrose gradient centrifugation (as in Fig. 1) followed first by heating 10 min 80 °C (as in Fig. 1*c*) and then by chromatography on DEAE-cellulose (as in Fig. 2). The figure shows a microdensitometer trace of a stained 15% SDS-polyacrylamide gel. *b-d*, Assay of the material from (*a*) for insertion of superhelical turns into relaxed DNA (as in Fig. 2*a*); *b*, in the presence of histones (extracted from mouse fibroblasts by 0.2 M H₂SO₄) and nicking-closing enzyme (purified as in legend to Table 1); *c*, as *b*, but histones omitted; *d*, as *b* but the purified nucleosome assembly protein (illustrated in Fig. 3*a*) was omitted. *e-g*, as *b-d* but using assembly protein (not shown) which had been prepared by incubating a heated sucrose gradient fraction with 100 μ g ml⁻¹ ribonuclease A and 150 unit ml⁻¹ micrococcal nuclease (in 2 mM CaCl₂, 50 mM NaCl, 10 mM Tris pH 8.0) for 2 h at 25 °C followed by chromatography on Sephadex G-75 (in 0.5 mM EDTA, 50 mM NaCl, 10 mM Tris pH 7.5) to remove oligonucleotides and the nucleases.

Figure 4 illustrates two other criteria which indicate that the protein described assembles nucleosomes. The sedimentation profile of the incubation product resembles that of naturally-occurring SV40 nucleohistone complex and contains approximately equal amounts of histones and DNA (Fig. 4*a*). In addition the incubation product has the characteristic beaded appearance of the SV40 minichromosome (Fig. 4*b*).

Nicking-closing enzyme is not required for nucleosome assembly. Although it is required to relax torsion so that the insertion of superhelical turns into DNA can be visualised in the gel assay, it is not required when nucleosome assembly is

Fig. 4 The protein of 29,000-dalton subunits causes DNA and histones to form a discrete nucleoprotein complex.

a. Velocity sedimentation of the nucleoprotein product obtained by incubating $0.1 \mu\text{g}$ ^3H -SV40 form I ($55,000 \text{ c.p.m. } \mu\text{g}^{-1}$) DNA (●) with $0.2 \mu\text{g}$ ^{35}S -labelled mouse fibroblast histones ($7,000 \text{ c.p.m. } \mu\text{g}^{-1}$) (○), in the presence of $20 \mu\text{l}$ of the heated peak fraction from a sucrose gradient (similar to the track marked by an arrow in Fig. 1c). After incubation for 3 h at 20°C without nicking-closing enzyme the product was centrifuged through a 10–30% sucrose gradient in 60 mM KCl, 1 mM EDTA, 10 mM Tris pH 7.5 for 70 min at 50,000 r.p.m. in a Beckman SW60Ti rotor. The arrow marked 'DNA' indicates the position of free SV40 DNA form I and the arrow marked 'DNP' indicates the position of authentic SV40 minichromosome from infected cells. **b.** Electron micrographs of the nucleoprotein product obtained by incubation in the conditions described in **a** followed by spreading and shadowing as described previously⁷. (Nicking-closing enzyme was included in the incubations illustrated in **b**, but it is not necessary for the formation of such structures.) $\times 130,000$.



monitored by sedimentation or electron microscopy. For these assay methods the purified assembly protein (as illustrated in Fig. 3a) is sufficient to convert histones and DNA into nucleosomes.

The protein which co-purifies with assembly activity binds histones to form an active complex

Figure 5 illustrates that the purified nucleosome assembly protein binds labelled histones. All four of the nucleosome core histones bind and co-sediment as a complex with the assembly protein (at approximately 8S). The complex sediments much faster than free histones and slightly, but reproducibly, faster than the free assembly protein. Coomassie blue staining of gels followed by microdensitometry and comparison with standard histone preparations from nuclei reveals that all four of the nucleosome core histones are present in the complex in approximately equimolar amounts. Histone H-1 has not been detected so far but this could be due to technical causes.

The formation of the complex between histones and the nucleosome assembly protein is unlikely to be a nonspecific, electrostatic adsorption artefact for several reasons. First, at low histone concentrations essentially all of the histones sediment as a uniform, discrete complex. Second, when the histone concentration is increased binding can be saturated without causing the complex to aggregate or sediment faster; instead the excess histones sediment as free histones. Third, the assembly protein masks sufficient positive charges on the histones to inhibit their binding to CM-cellulose at neutral pH. Fourth, if small amounts of labelled histones are added to crude egg homogenates the added histones sediment discretely as the complex described here, indicating that the vast excess of cellular proteins does not compete effectively for histone binding against the nucleosome assembly protein, even though several of the other cellular proteins are more acidic than the assembly protein. Fifth, when the complex formed between the purified assembly protein and labelled histones is isolated from a sucrose gradient and incubated with DNA the histones are transferred to the DNA with the formation of a beaded nucleoprotein complex which contains supercoiled DNA.

Cross-linking studies should provide further information on the specificity and stoichiometry of histone binding.

Properties of the nucleosome assembly protein

The protein which co-purifies with nucleosome assembly activity electrophoreses as a rather broad band with an apparent MW of 29,000 in SDS-polyacrylamide gels. When it is electrophoresed over long distances it resolves into two distinct bands, suggesting that the band seen in Figs 1–3 probably

contains more than one polypeptide chain. Its sedimentation and gel filtration properties suggest that the native protein is larger than 100,000 daltons. It does not bind to CM-cellulose above pH 6 but binds to DEAE-cellulose and elutes between 0.3 M and 0.4 M KCl at pH 7.5, suggesting that it is strongly

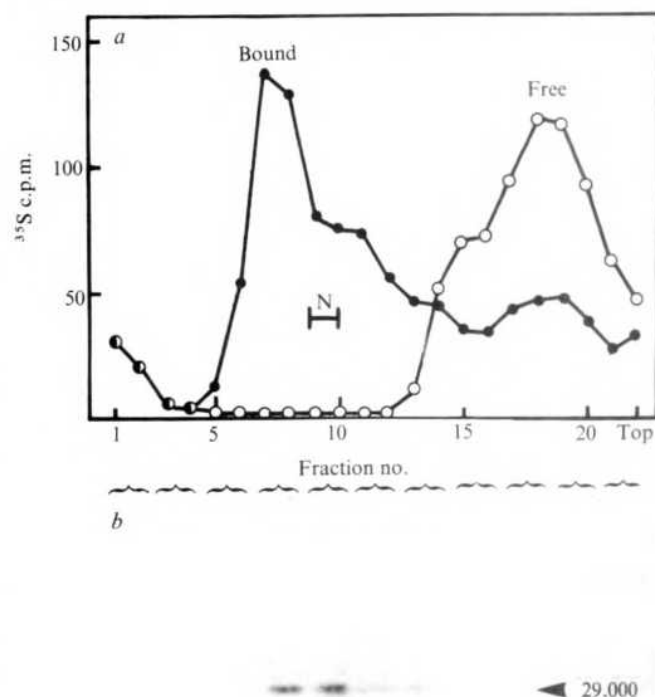


Fig. 5 The nucleosome assembly protein binds histones. **a.** distribution of ^{35}S -methionine labelled, acid-extracted mouse histones after centrifugation through a 10–30% sucrose gradient. ●, Histones were incubated with a $10\times$ concentrated (Minicon B-15) heated sucrose gradient fraction (as marked by arrow in Fig. 1c) with 50 mM NaCl for 20 min before centrifugation in a separate tube from the free histone marker (○). Radioactivity was measured by liquid scintillation counting 50- μl aliquots of each fraction in Aquasol. Fractions from (a) (●) were pooled in pairs and 50- μl aliquots were electrophoresed in a 15% polyacrylamide gel (as in Fig. 1a) to locate the polypeptide of MW 29,000 by Coomassie blue staining. (At the histone concentration used in this figure histone bands were stained too faintly for photographic reproduction.) The bracket marked N indicates the position of the nucleosome assembly protein when it is centrifuged in the absence of histones (fractions 9 and 10). Centrifugation was for 20.5 h at 50,000 r.p.m. in a Beckman SW60Ti rotor at 4°C . Gradients contained in 2mM EDTA, 20mM Tris-HCl pH 7.5.

acidic. This conclusion is confirmed by isoelectric focusing. Thus on two dimensional gels¹¹ the protein has an isoelectric point of 5.

Additional evidence that this protein is indeed responsible for nucleosome assembly comes from the observation that it shows the same remarkable resistance to thermal denaturation and proteolytic degradation as the assembly activity. Thus its sedimentation as a multi-subunit structure and its ability to bind histones are not affected by heating. After prolonged treatment with chymotrypsin the protein's sedimentation constant is only slightly decreased and it continues to bind histones. Electrophoresis reveals that at least one cut has occurred in each polypeptide chain, since the band of MW 29,000 is replaced by two large fragments of MW approximately 26,000 but which still sediment as a multi-subunit structure. Proteinase K at pH 10 which abolishes the assembly activity also cleaves the protein, generating fragments of less than 20,000 daltons.

Five ml of egg homogenate (3 ml packed eggs + 2 ml buffer) containing 1.25 g total protein yields 160 µg of pure protein. 1 µg of pure protein can convert at least 0.25 µg DNA into nucleosomes in the presence of 0.5 µg histones.

The nucleosome assembly process

When histones and DNA are mixed in solution at physiological ionic strength, they instantly form a precipitate. When the protein described here is mixed with histones before addition of DNA, precipitation is prevented and nucleosomes form. We observed previously that the egg's histone pool is complexed to a large negatively charged molecule and that fractions containing this complex interact with DNA to form nucleosomes⁷. Therefore we proposed that histones are organised into nucleosome precursor complexes by an acidic factor which has a 'catalytic' role in the assembly process⁷. The protein we have purified clearly binds histones and transfers them to DNA to form nucleosomes. The possibility that it is recycled remains to be tested.

Nucleosomes can be assembled by prolonged dialysis from 2 M NaCl (reviewed in ref. 2). Therefore it seems that the spatial information required for histone-histone interactions and histone-DNA interactions resides in the histones and DNA themselves. We suggest that the role of the protein we have purified is that of a 'molecular chaperone' which prevents incorrect ionic interactions between histones and DNA. Thus by binding histones and neutralising their positive charges the

nucleosome assembly protein could prevent nonspecific ionic interactions and allow only selected specific interactions to occur. The additional possibility that specific interactions between different histones occur in the complex formed with the nucleosome assembly protein remains to be tested.

The nucleosome assembly reaction studied here seems to have very simple requirements. It requires only the protein preparation illustrated in Fig. 3a, DNA and acid extracted (or salt extracted) histones incubated in NaCl (or KCl) concentrations between 50 mM and 150 mM. It is inhibited by higher NaCl concentrations. The reaction will proceed to completion in 3 h at 0°C. We have been unable to find requirements for low molecular weight co-factors, such as Mg²⁺ or ATP.

Eggs of *Xenopus laevis* are unusual in that they divide to form 5,000 cells in 5 h with some cell cycles of only 10 min. In addition they accumulate a large histone pool, sufficient for 15,000 nuclei⁴⁻⁶. Therefore it is possible that the nucleosome assembly protein we have described is only an adaptation to this rapid rate of chromosomal replication. However, during the course of this work we have found that mouse fibroblast nuclei and wheat embryos also contain a thermostable protein which converts histones and DNA into nucleosomes (B.M.H., A.D.M. & R.A.L., in preparation). Therefore it will be interesting to determine if the protein we have described is a universal mechanism for assembling the nucleosome subunits of eukaryotic chromatin.

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Promoter-specific inhibition of transcription by antibiotics which act on DNA gyrase

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Nalidixic acid, novobiocin and coumermycin specifically inhibit phage promoter-dependent transcription of the trp operon in ϕ 80ptp but not transcription from the authentic trp promoter. The nalidixic acid inhibition is not observed in an E. coli strain containing a nalA^r mutation. These results indicate that DNA gyrase is involved in transcription.

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DNA GYRASE catalyses the ATP dependent introduction of negative superhelical twists into double stranded closed circular DNA in *Escherichia coli*¹. This activity is required for λ integrative recombination² and replication of *E. coli*^{3,4}, plasmids^{5,6} and bacteriophages⁷⁻⁹. DNA gyrase is inhibited by the antibiotics coumermycin and novobiocin¹⁰. The *cou* locus mapping close to the *dnaA* at 82 min on the *E. coli* map determines resistance to these drugs^{11,12}.

DNA gyrase activity is also inhibited by nalidixic acid (NAL) and oxolinic acid^{13,14}. Resistance to these drugs is determined by

the *nalA* locus mapping at 48 min on the *E. coli* map¹⁵. It is thought that the nick closing activity required in the supercoiling reaction resides in the NAL target protein, *pnaI*, because (1) the ATP independent DNA gyrase catalysed relaxation of supercoiled DNA is inhibited by NAL and oxolinic acid but not by novobiocin or coumermycin and (2) incubation of DNA gyrase and DNA with NAL or oxolinic acid results in the formation of a relaxation type complex.

At low doses NAL is thought to be a specific inhibitor of DNA replication in *E. coli*. However at higher doses several workers have observed inhibition of transcription¹⁶⁻¹⁸. In preliminary experiments we observed indications that NAL interfered with $\phi 80$ phages transcription at doses normally thought to inhibit only DNA replication. Here we report the effect of NAL on transcription of the tryptophan (*trp*) operon integrated into the $\phi 80$ phage genome.

Nalidixic acid is a promoter-specific inhibitor of *trp* expression

When the bacterial *trp* operon is integrated into the λ phage genome, transcription of the operon may initiate at either the authentic *trp* promoter (P_{trp}) or at the leftward phage promoter as shown in Fig. 1^{19,20}. Initiation of transcription from P_{trp} is turned off in the presence of tryptophan and the *trp* repressor. Read-through transcription of the *trp* operon from the leftward phage promoter can occur in the presence of the *trp* repressor but is completely inhibited when phage repressor molecules are present. Subsequent studies yielded identical results with $\phi 80ptrp$ phages^{21,22}.

Infection of a nonlysogenic *E. coli trpE*⁻ strain (MO1512) with bacteriophage $\phi 80ptrp$, in the presence of tryptophan, led to immediate synthesis of anthranilate synthetase (ASase; product of the *trpED* genes) as shown in Fig. 2a. ASase synthesis occurred by the read-through transcription of the *trp* operon since no phage repressor molecules were present in the host cell. However, when the $\phi 80ptrp$ infected cells were incubated in the presence of 40 $\mu\text{g ml}^{-1}$ NAL, no ASase activity was detected even after prolonged incubation.

The effect of NAL on ASase synthesis originating at the P_{trp} promoter is shown in Fig. 2b. In this experiment *E. coli* strain MO1513 *trpE*⁻ ($\phi 80^+$) was infected with $\phi 80ptrp$ in the absence of tryptophan. ASase synthesis, due to transcription from the authentic *trp* promoter, began immediately after removal of tryptophan from the culture medium, as shown by the control cells in Fig. 2b. When NAL was present only a slight decrease in ASase activity was seen throughout the incubation period. Dose-response curves for a 60-min incubation are shown in Fig. 3a. Concentrations of NAL which virtually eliminate ASase synthesis originating from the phage promoter have essentially no effect on synthesis originating from P_{trp} .

Nalidixic acid inhibition occurs in the absence of DNA replication

The amount of ASase synthesised in the procedure used in Figs 2 and 3 is regulated by the number of $\phi 80ptrp$ templates available for transcription²². Thus, it was possible that NAL inhibited ASase synthesis simply by interfering with phage replication. Therefore, the effect of NAL on phage promoter dependent ASase synthesis was examined in the absence of DNA replication. An *E. coli* strain lacking DNA polymerase I and II and containing a temperature-sensitive DNA polymerase III (*polA*⁻, *polB*⁻, *polC*_{ts}) was infected with $\phi 80ptrp$ and treated with NAL. As shown in Fig. 4a, addition of NAL to $\phi 80ptrp$ infected cells incubated at 30°C led to rapid partial inhibition of ASase synthesis by the read-through mechanism. Addition of NAL to $\phi 80ptrp$ -infected cells incubated at the restrictive temperature also led to inhibition of phage promoter controlled ASase synthesis (Fig. 4b). However, somewhat less inhibition was observed at the restrictive temperature than at the permis-

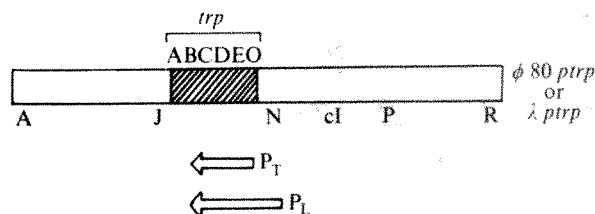


Fig. 1 Transcriptional map of bacteriophages $\phi 80$ and λ carrying the *E. coli trp* operon. P_T , authentic *trp* promoter (repressed by tryptophan but not by phage repressor); P_L , early leftward phage promoter (repressed by the phage repressor but not by tryptophan).

sive temperature. This indicates a small portion of the NAL inhibition may arise from interference with replication but most of the effect is replication independent.

Nalidixic acid inhibits phage promoter-directed *trp* transcription

DNA-RNA hybridisation experiments were conducted to determine whether NAL inhibition was occurring at the level of transcription or translation. The results of these experiments are summarised in Table 1. Addition of NAL to $\phi 80ptrp$ infected cells reduced the amount of phage promoter-controlled *trp* mRNA synthesis to about 10% of the level observed in the absence of the drug. On the other hand, the drug caused a slight increase in the amount of mRNA derived from the P_{trp} promoter. The effects observed with chloramphenicol are very different. Chloramphenicol specifically inhibits P_{trp} transcription but does not affect phage transcription. Similar chloramphenicol effects have been observed in $\lambda ptrp$ phages^{23,24}.

We considered the possibility that NAL was stimulating degradation of the phage promoter initiated mRNA rather than inhibiting mRNA synthesis. The effect of NAL on the stability of preformed mRNA is shown in Fig. 5. In these experiments rifampicin was added at the time of addition of NAL to inhibit mRNA initiation. NAL did not significantly increase the rate of *trp* mRNA degradation.

At low concentrations NAL inhibits DNA replication^{18,25,26}. At these concentrations transcription is not inhibited as judged both by incorporation of labelled precursor into RNA^{18,25,26} and by direct examination of several bacterial operons¹⁷. Based on

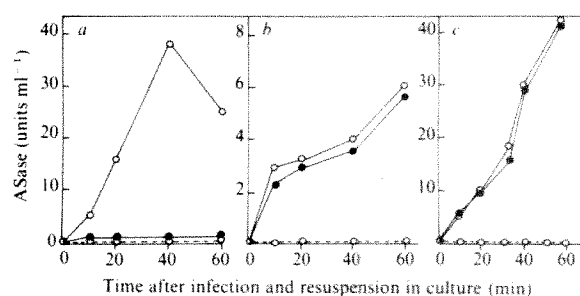


Fig. 2 Nalidixic acid inhibition of ASase synthesis directed by a $\phi 80ptrp$ template. (a) Read-through transcription from the phage promoter in a *nalA*⁺ strain. In the presence of tryptophan, strain MO1512 *trpE*⁻ was grown to a density of 2×10^8 cells ml^{-1} , infected with $\phi 80ptrp$ and treated with 40 $\mu\text{g ml}^{-1}$ NAL (●). (b) Transcription from the authentic *trp* promoter in a *nalA*⁺ strain. In the absence of tryptophan, strain MO1513 *trpE*⁻ ($\phi 80$) was grown to a density of 2×10^8 cells ml^{-1} and in the absence of tryptophan, infected with $\phi 80ptrp$ and treated with 40 $\mu\text{g ml}^{-1}$ NAL (●). (c) Read-through transcription from the phage promoter in a *nalA*⁻ strain. In the presence of tryptophan, strain MO15124 *trpE*⁻ *nalA26* was grown to a density of 2×10^8 cells ml^{-1} , infected with $\phi 80ptrp$ and treated with 40 $\mu\text{g ml}^{-1}$ NAL (●). Controls: in all cases were—infected with $\phi 80ptrp$ but not treated with NAL (○—○); not infected with $\phi 80ptrp$ and not treated with NAL (○—○). Experimental procedures are described in full elsewhere²³.

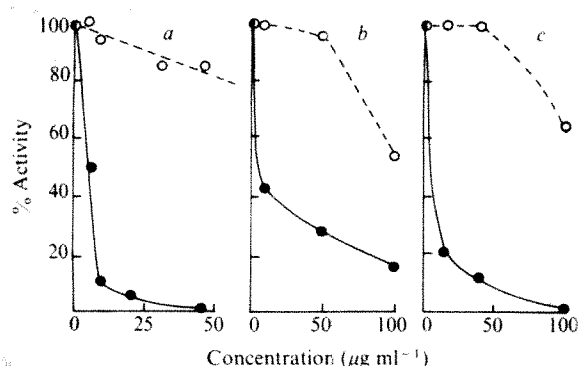
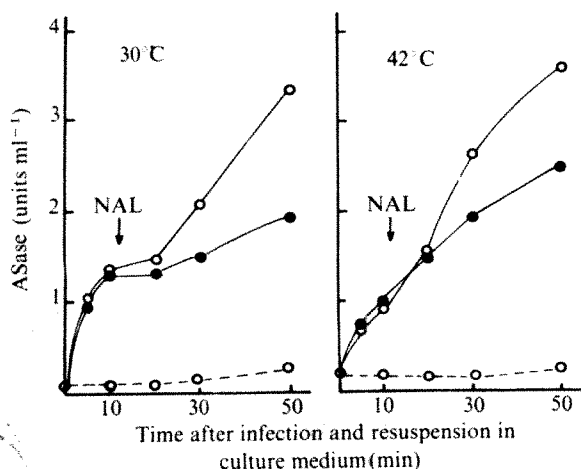


Fig. 3 Inhibition of ASase synthesis by various concentrations of nalidixic acid (a), novobiocin (b) and coumermycin (c). The per cent activity was calculated from the amount of ASase activity observed at 60 min at the indicated dose. 100% activity was 39 units ml⁻¹ for phage directed synthesis (●) and 6.7 units ml⁻¹ for P_{trp} directed synthesis (○). Experimental procedures are described in Fig. 2.

these results it has been assumed that NAL is a specific inhibitor of DNA replication. However, our results show that transcription monitored by the production of competent mRNA and also by enzyme synthesis can be inhibited by the same NAL doses previously believed only to inhibit DNA replication. Therefore, NAL should not be used as a specific inhibitor of DNA replication.

In 1973, Puga and Tessman¹⁶ reported that nalidixic acid interfered with plasmid phage S13 mRNA synthesis at drug levels which did not affect host mRNA synthesis. Since all S13 mRNA synthesis was equally inhibited and it had been previously observed that intercalating dyes inhibited transcription²⁷, these authors proposed that the specificity of the inhibition was due to a preferential intercalation of the antibiotic into the supercoiled plasmid DNA. However, our results show that NAL inhibits, with total specificity, one of the two promoters both of which serve the same operon and are on the same

Fig. 4 Nalidixic acid inhibition of phage promoter dependent ASase synthesis in the presence (30 °C) and absence (42 °C) of DNA replication. Strain BT10266 *polA*⁻, *polB*⁻, *polC*_{ts} was grown to 2 × 10⁸ cells ml⁻¹ by incubation at 30 °C in minimal medium containing tryptophan²³. Following infection with ϕ80_{trp} the culture was divided into two portions, one of which was further incubated in fresh minimal medium at 30 °C while the other was incubated at 42 °C. After 15 min, 40 μg ml⁻¹ NAL was added to portions of each of the cultures and the incubation at the respective temperature continued (●—●). Controls: infected with ϕ80_{trp} but not treated with NAL (○—○); not infected with ϕ80_{trp} and not treated with NAL (○—○—○).



plasmid molecule. Therefore, the simple notion that superhelical density generates NAL specificity must be rejected.

In experiments shown in Fig. 4 only partial inhibition of phage promoter controlled ASase synthesis was observed. This is in contrast to the complete inhibition observed in the experiment shown in Fig. 2. In the former experiment NAL was added after initiation of transcription whereas in the latter experiment it was added just before. However, the former experiment also involved a multiple mutant strain while the latter involved a wild-type strain. To determine which of these factors was significant NAL was added to the same wild-type strain used for Fig. 2 but after transcription was initiated. A partial inhibition of ASase synthesis was seen just like that shown in Fig. 4 (data not shown). As the effect of NAL is greater when added before transcription initiates, it is likely that NAL acts preferentially on initiation.

DNA gyrase is involved in transcription

Mutations at the *nalA* locus confer NAL resistance to DNA synthesis^{18,27}. To see if this same locus was involved in NAL sensitive transcription we examined ASase synthesis in a derivative of *E. coli* strain MO1512 containing the *nalA26* mutation (Fig. 2c). No NAL inhibition of phage promoter directed synthesis was observed in the *nalA*^r *E. coli* strain in conditions where total inhibition is seen in the isogenic *nalA*^s strain (Fig. 2a). This shows the NAL-target protein involved in transcription is the same protein involved in DNA replication.

The recently isolated *nalA* protein participates in DNA gyrase mediated reactions^{1,10,13,14}. Since this enzyme is also inhibited by coumermycin and novobiocin, we examined the

Table 1 Nalidixic acid inhibition of *trp* mRNA synthesis

	Anti-biotic	Tryptophan (50 μg ml ⁻¹)	<i>trp</i> mRNA (% of total RNA)	Specific activity of ³ H-RNA (c.p.m. per mg)
Phage promoter directed synthesis (strain AE1)	None	+	1.17	5.8 × 10 ³
	NAL	+	0.12	6.3 × 10 ³
	CAP	+	3.01	5.6 × 10 ³
P _{trp} directed synthesis (strain AE1 (h ⁺ i ⁸⁰))	None	—	3.02	3.3 × 10 ³
	NAL	—	3.48	1.3 × 10 ³
	CAP	—	0.18	3.0 × 10 ³

E. coli strain *trpAE1* or its lysogen *trpAE1* (h⁺i⁸⁰) was infected with ϕ80_{trp} phages and incubated at 30 °C in the presence or absence of L-tryptophan (50 μg ml⁻¹), respectively. NAL (40 μg ml⁻¹) or chloramphenicol (CAP; 100 μg ml⁻¹) was added at the 8th minute of incubation after the phage infection. Pulse-labelling was carried out with 100 μCi of ³H-uridine for 1 min starting 29.5 min after infection. The value of *trp* mRNA hybridised with λ_{trp} DNA is expressed as a percentage of total labelled RNA added into a hybrid assay system and is the average of duplicate determinations. The value of λ DNA background was subtracted from each hybrid value. Details for procedures used in these experiments are described elsewhere³⁹.

effect of these antibiotics on ASase synthesis. The inhibition of P_{trp} and phage promoter directed ASase synthesis by different doses of novobiocin and coumermycin (Fig. 3b, c) show a striking parallel with NAL inhibition. In all cases P_{trp} directed synthesis is much less sensitive to these antibiotics than phage promoter directed ASase synthesis. The 20% inhibition of P_{trp} directed ASase synthesis at 100 μg ml⁻¹ novobiocin probably reflects inhibition of DNA replication observed at this dose level in this strain (see ref. 28). The greater inhibition observed with coumermycin could be due to the greater permeability of *E. coli* to this antibiotic¹¹.

Our results provide the first direct evidence that DNA gyrase is involved in transcription. Three other antibiotics known to be specific inhibitors of DNA gyrase mediated supercoiling dis-

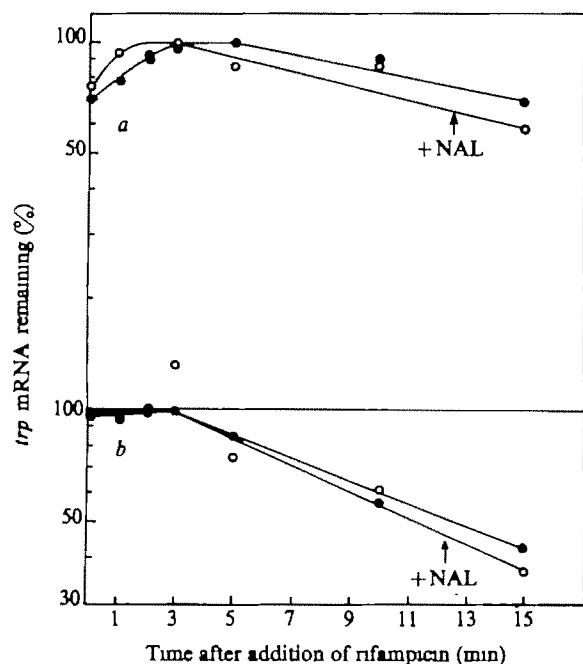


Fig. 5 Effect of nalidixic acid on the stability of *trp* mRNA synthesised from a phage promoter (a) and the authentic *trp* promoter (b). At 30 °C strain *trpAEl* (a) or strain *trpAEl* (h⁺) (b) was infected with $\phi 80trp$ in the presence of (a) or absence of (b) L-tryptophan (50 $\mu\text{g ml}^{-1}$), respectively. Pulse-labelling (1 min) carried out with 33 $\mu\text{Ci ml}^{-1}$ ³H-uridine at the 15th minute after the phage infection was immediately followed by addition of rifampicin (300 $\mu\text{g ml}^{-1}$) in the presence of (○) or absence (●) of 40 $\mu\text{g ml}^{-1}$ NAL. At the times indicated portions of the cultures (6×10^9 cells) were removed for RNA extraction. The *trp* mRNA values are expressed as ³H-RNA hybridised per μg RNA and normalised to 100% for the maximum value. The value represented is the average of duplicate determinations. Detailed procedures are described elsewhere³⁹.

played the same promoter specific inhibition of transcription as NAL. The specificity implies that transcription requires DNA gyrase when initiation occurs at the phage promoter but not when initiation occurs at the *trp* promoter. In addition, it seems that it is the supercoiling activity of DNA gyrase that is involved in transcription. The effectiveness of these inhibitors on phage promoter-directed transcription is striking when compared to the inhibitor concentration required to obtain an equivalent degree of inhibition of DNA synthesis.

The origin of the specific requirement of DNA gyrase for one of the two promoters remains unexplained. Botcham²⁹ observed an increase in early phage promoter mRNA synthesis in λ when supercoiled DNA template was used instead of a relaxed template. Although no studies have directly compared *P*_{trp} transcription on supercoiled and relaxed templates, it is possible that by raising the superhelix density DNA gyrase could preferentially assist a weak promoter at binding RNA polymerase. This simple mechanism would be consistent with suggestions that complex formation between RNA polymerase and a promoter is accompanied by duplex unwinding^{30,31}. However, the DNA gyrase-requiring promoter seems to be stronger than the *P*_{trp} promoter when total enzyme activity is examined. It is difficult to explain this simply by considerations of superhelix density.

As an alternative it is tempting to speculate that the critical factor in determining the requirement for DNA gyrase is the linking of transcription with translation^{23,24}. The phage promoter which requires DNA gyrase is not linked to translation whereas the gyrase-independent promoter is linked to translation. Clearly, further studies are needed to determine whether

it extends to other host cell translational-independent promoters.

Another alternative hypothesis is that the phage promoter is subject to some type of topological constraint which DNA gyrase must overcome in order for transcription to occur. For example the phage promoter could be involved in a loop of DNA where the ends were held rigid by attachment to a cell constituent such as the cell membrane. Such a loop structure had been postulated for the *E. coli* chromosome^{32,33}.

In λ phage it has been reported that transcription initiated at the leftward phage promoter is dependent on the phage N protein³⁴⁻³⁷. This protein interacts with RNA polymerase and DNA so that in the presence of the rho termination factor, the RNA polymerase no longer recognises a termination point present in the phage genome³⁸. Transcription of the *trp* operon in $\phi 80$, is dependent on the phage N gene product, as the operon is integrated into the phage at a point past the rho termination site. Therefore, it is possible that NAL is acting as an anti-N effector, so that phage transcription in the presence of the drug is terminated at the rho site. However, in preliminary experiments, NAL inhibition of phage controlled ASase synthesis has been observed in $\lambda ptrp$ phages lacking the rho dependent termination site (data not shown).

Although it is not clear how *pnaI* participates in RNA synthesis, it is obvious that the transcriptional apparatus at some operons is dependent on the *pnaI* protein. Thus, NAL and *pnaI* can serve as a probe to examine the transcriptional process at least in the case of phage promoter synthesis and perhaps in bacterial transcription.

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Bacteriophages inhibit degradation of abnormal proteins in *E. coli*

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On infection of Escherichia coli cells by bacteriophages T4, T5 or T7, the degradation of E. coli protein fragments and abnormal proteins is inhibited. Normal E. coli proteins, however, continue to be degraded at their usual rates. T4 early protein(s) is needed to inhibit the turnover of abnormal proteins in T4-infected E. coli cells.

HALF LIVES of proteins with abnormal conformations and protein fragments are much shorter than those of most normal proteins in bacterial and in eukaryotic cells^{1,2}. Most nonsense fragments of *Escherichia coli* proteins are so short-lived that either they fail to accumulate to detectable levels or they are found in much lower quantities than wild-type proteins^{3,4}. *E. coli* polypeptides containing missense alterations also tend to turn over rapidly compared to normal proteins⁵. Nonsense fragments of virus proteins from bacteriophage T4-infected *E. coli* cells are, in contrast, often found at levels approximating those of normal T4 proteins⁶. In this article we present evidence relating to the basis for this difference in stability between *E. coli* and T4 protein fragments.

The identities and functions of the specific enzymes responsible for the rapid turnover of abnormal proteins and protein fragments are not known in detail, but some information of interest is available about this system in *E. coli*. The degradation of such proteins is ATP-dependent². If cellular ATP levels are substantially reduced, the turnover of abnormal proteins and protein fragments is also reduced⁷⁻⁹. To investigate the characteristics of this degradative system, the intracellular levels of abnormal proteins can be increased by treating cells with puromycin, leading to the production of protein fragments, or with amino acid analogues, leading to the production of full-sized proteins with abnormal conformations. In *E. coli*, both puromycyl peptides and analogue-containing proteins are degraded by an ATP-dependent system^{2,7,8}, as are other abnormal proteins and protein fragments. Specific mutations called *deg*⁻ (or *lon*⁻) at 11.4 minutes on the *E. coli* map, near *proC*, reduce the turnover rates of protein fragments¹⁰, abnormal proteins, and also of phage λ proteins containing temperature-sensitive, missense alterations¹¹. The molecular basis for the effect of *deg*⁻ mutations on the *E. coli* degradation system is obscure.

The mechanism by which the *E. coli* degradation system recognises abnormal proteins is also obscure. Electron micrographs suggest that abnormal proteins, if present in sufficient quantity, form intracellular aggregates which appear adjacent to the *E. coli* inner membrane^{12,13}. The ATP-dependent degradative activity is probably bound to the *E. coli* cell membrane (R. Voellmy and A. L. Goldberg, personal communication). The adherence of abnormal proteins to the cell membrane, probably through exposed hydrophobic regions of the proteins, may provide the recognition signal for the degradation system. It may be of interest, therefore, that the earliest detectable step in T4 capsid assembly involves the formation of amorphous aggregates of unprocessed capsid proteins on the cell membrane¹⁴. These aggregates are morphologically indistinguishable from those formed by abnormal *E. coli* proteins¹. Yet, if these T4

proteins were degraded, the production of progeny virions could not occur. The mechanism by which T4 proteins probably avoid the *E. coli* degradation system is described below.

Degradation of *E. coli* proteins and puromycyl peptides after T4 infection

To study the effects of bacteriophage infection on the degradation of abnormal *E. coli* proteins and protein fragments, we first examined the degradation of puromycyl protein fragments (PPF) in uninfected and in T4-infected *E. coli* CR63 cultures. We exposed a CR63 culture to ¹⁴C-leucine in the presence of puromycin; the unincorporated puromycin and ¹⁴C-leucine were then removed and the cells were resuspended in fresh medium with unlabelled leucine. The culture containing the ¹⁴C-PPF was divided into two equal volumes. T4 particles in tryptone broth were added to one volume of cells and sterile broth was added to the other. Samples taken before the culture was divided and at various times thereafter were added to cold trichloroacetic acid (TCA) at a final TCA concentration of 5% to precipitate high molecular weight polypeptides. As ¹⁴C-PPF are degraded to amino acids which are soluble in TCA the corresponding radioactivity appears in the TCA supernatant. The fraction of the ¹⁴C-leucine that was TCA-insoluble before dividing the culture, but that became TCA-soluble afterwards is referred to as the percentage of protein degraded.

The amount of protein degraded in the uninfected cultures varies as a function of the puromycin concentration used during the labelling period (Fig. 1). When no puromycin is added to the culture, 4% of the ¹⁴C-labelled proteins is degraded in 30 min; in the presence of 15 $\mu\text{g ml}^{-1}$, puromycin protein degradation in 30 min increases slightly to 5%; 50 $\mu\text{g ml}^{-1}$ puromycin produces a level of 14% protein degradation; and 100 $\mu\text{g ml}^{-1}$ puromycin 24%. The correlation between increasing concentrations of puromycin and increasing levels of protein degradation is expected; as the concentration of puromycin is increased there is an increase in the production of protein fragments that are turned over rapidly relative to normal proteins that are turned over slowly.

Although bacteriophage T4 infection has no detectable effect on the degradation of normal *E. coli* proteins (Fig. 1a), T4 infection drastically inhibits the degradation of ¹⁴C-PPF (Fig.

Table 1 PPF degradation after infection by T4, T5 or T7 phages

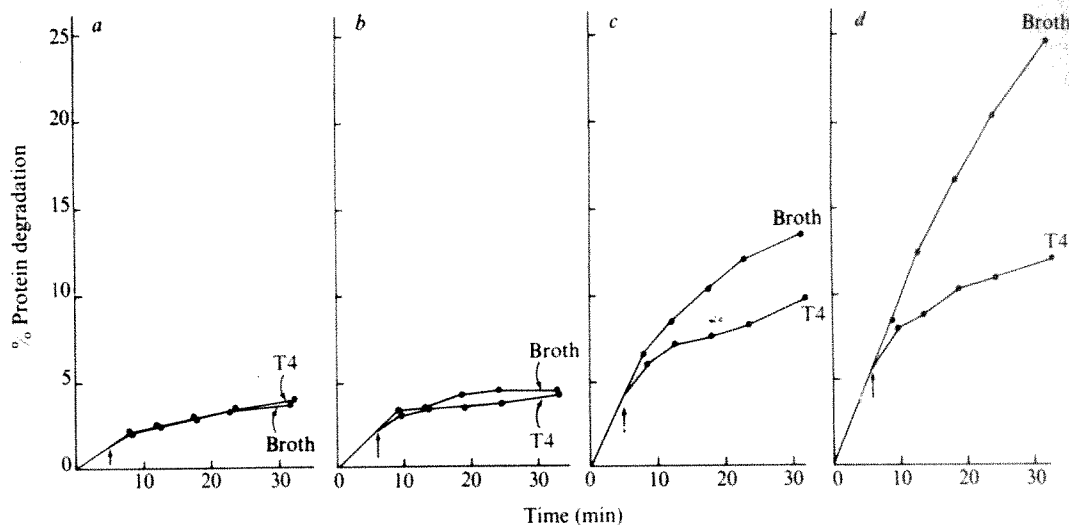
Culture*	% Protein degraded†
Uninfected	14
T4-infected	9
T5-infected	10
T7-infected	11

* *E. coli* B cultures growing at 37°C.

† The % of PPF degraded during the first 15 minutes after infection (or the comparable time interval for the uninfected control). Normalised data from two experiments are presented. Comparisons of PPF degradation at later time intervals are precluded by the short T7 latent period.

Fig. 1. Degradation of normal proteins and of puromycin protein fragments (PPF) in uninfected and in T4-infected *E. coli* CR63 cells. Puromycin ($0\text{--}100\text{ }\mu\text{g ml}^{-1}$) was added to mid-log phase CR63 cultures (5×10^8 cells per ml) in M9 medium¹⁶; 10 min later ^{14}C -leucine ($0.2\text{ }\mu\text{Ci ml}^{-1}$) was added. After a 5 min labelling period, the bacteria were collected on Millipore filters and washed twice to remove unincorporated puromycin and ^{14}C -leucine. The bacteria were then resuspended in fresh medium containing

unlabelled leucine ($300\text{ }\mu\text{g ml}^{-1}$). The cultures were divided and one half of each was infected with wild type T4 D particles. Unless otherwise stated, the multiplicity of infection in this and in other experiments was 5 phage per cell, and the T4 particles used were wild type T4 D. Samples taken just before the cultures were divided (our 0-time samples) and at various times thereafter were added to cold TCA at a final TCA concentration of 5%. The TCA insoluble and soluble materials were separated from each other by low speed centrifugation. The amounts of ^{14}C -leucine in the TCA supernatants and pellets were determined by liquid scintillation counting techniques. The pellets were dissolved with Soluene (Packard) and then counted in BBOT scintillation fluid; aliquots of the supernatants were counted in Patterson Greene scintillation fluid⁷. The fraction of the ^{14}C -leucine that was TCA insoluble before dividing the culture, but that became TCA soluble afterwards is plotted as the percent of protein degradation. The arrows in this and in subsequent figures indicate the times when phages or other treatments were added to the bacterial cultures. *a*, No puromycin; *b*, $15\text{ }\mu\text{g ml}^{-1}$; *c*, $50\text{ }\mu\text{g ml}^{-1}$ and *d*, $100\text{ }\mu\text{g ml}^{-1}$ puromycin.



1*b*, *c*, *d*). Thus the degradation to acid soluble material of proteins made in the absence of puromycin is quantitatively similar in the T4-infected and in the uninfected cultures. As the concentration of puromycin increases, causing the production of progressively more PPF, the T4-induced inhibition of PPF degradation becomes apparent.

Degradation of normal and canavanine-containing proteins after T4 infection

To determine whether T4 infection inhibits the degradation of abnormal proteins other than puromycin protein fragments and to extend our observations of normal protein turnover after T4 infection, we performed the following double label experiment. Normal proteins in *E. coli* A-33, an arginine auxotroph, growing in the presence of arginine were labelled with ^3H -lysine; after a suitable labelling period, unincorporated ^3H -lysine and arginine were removed, and the growth medium was supplemented with unlabelled lysine and L-canavanine; 5 min later ^{14}C -leucine was added to label the abnormal canavanine-containing *E. coli* proteins (CCP). At the end of the labelling period, the cells were collected, washed free of unincorporated canavanine and ^{14}C -leucine, and then resuspended in medium containing arginine and unlabelled leucine. Degradation of the ^3H -normal proteins and ^{14}C -abnormal canavanine-containing proteins was measured in the T4-infected and in uninfected control cultures. The results of this experiment (Fig. 2) show no detectable difference in the degradation of normal ^3H -proteins between the T4-infected and uninfected cultures. Yet, in the same cells the degradation of abnormal ^{14}C -proteins is severely inhibited in the T4-infected culture compared with the uninfected control. We conclude that during T4 infection, the rapid degradation of abnormal proteins and protein fragments is inhibited, but the turnover of normal proteins is unaffected.

Inhibition of protein degradation after infection by various multiplicities of T4 particles

If the processes of T4 adsorption and injection were themselves directly responsible for altering the cell in such a way that the degradation of PPF and CCP were inhibited, then the extent of inhibition of degradation might be expected to increase with

increasing multiplicities of infection (MOI). If, on the other hand, T4 proteins synthesised after phage adsorption and injection were responsible for inhibition of degradation, little change in inhibition would be expected from different MOIs since only about four infecting T4 genomes per cell can be expressed in normal conditions.¹⁷ We therefore infected

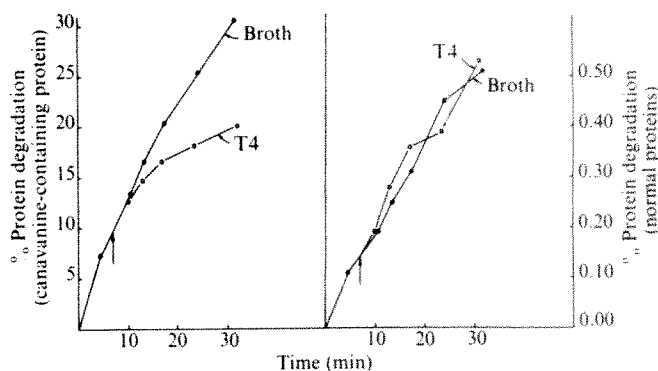


Fig. 2 The degradation of proteins in uninfected and in T4-infected *E. coli* A-33 cells containing both ^3H -normal proteins and ^{14}C -abnormal proteins. Normal proteins in *E. coli* A-33 bacteria growing in M9 medium supplemented with L-arginine ($50\text{ }\mu\text{g ml}^{-1}$) and L-tryptophan ($50\text{ }\mu\text{g ml}^{-1}$) were labelled with ^3H -lysine ($1\text{ }\mu\text{Ci ml}^{-1}$); the cells were then collected on filters, washed free of ^3H -lysine and of arginine, and resuspended in M9 medium lacking arginine, but supplemented with L-canavanine ($20\text{ }\mu\text{g ml}^{-1}$) and unlabelled lysine ($300\text{ }\mu\text{g ml}^{-1}$). After 10 min ^{14}C -leucine ($0.2\text{ }\mu\text{Ci ml}^{-1}$) was added to label the abnormal CCP; 5 min later the cells were again collected on filters, washed, and resuspended in fresh M9 with arginine ($100\text{ }\mu\text{g ml}^{-1}$) and unlabelled leucine ($300\text{ }\mu\text{g ml}^{-1}$). Thus, the *E. coli* A-33 culture contained ^3H -normal proteins and ^{14}C -CCP. One half of the culture was infected with T4 particles; degradation of ^3H -normal proteins and ^{14}C -CCP was assayed as described in Fig. 1. The low level of degradation of ^3H -normal proteins observed in this experiment is a consequence of the elapsed time between labelling normal proteins and the onset of our degradation assays. Those normal proteins that turn over rapidly had been degraded during the labelling of CCP and, therefore, had become TCA soluble by the start of our protein degradation measurements.

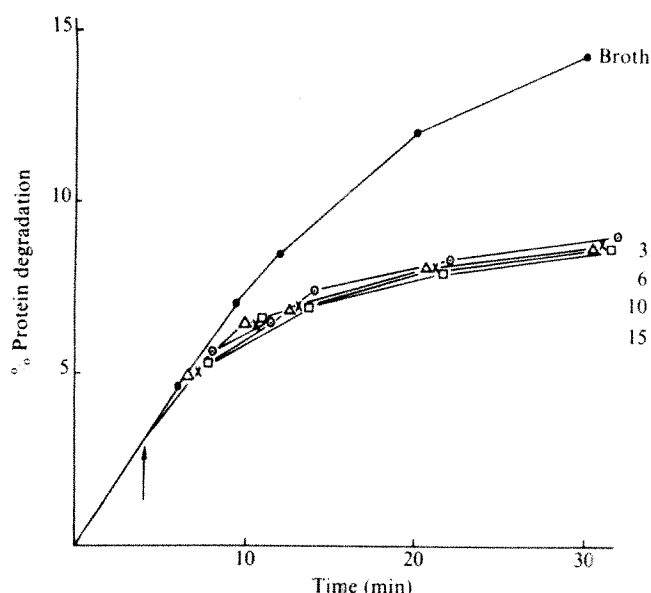


Fig. 3 The effect of various multiplicities of T4 particles on the degradation of *E. coli* puromycyl protein fragments. This experiment is similar to that in Fig. 1d, except that after exposure to puromycin ($100 \mu\text{g ml}^{-1}$) the CR63 culture was split into several aliquots which were then infected with the indicated multiplicities of T4 phages.

aliquots of a CR63 culture containing ^{14}C -PPF with various multiplicities of T4 particles. As can be seen in Fig. 3, the inhibition of PPF degradation following T4 infection is not multiplicity dependent. The same level of inhibition was observed with MOIs of 3, 6, 10, and 15 T4 particles per bacterium. Thus, it seems that the processes of T4 adsorption and injection are not directly responsible for the inhibition of PPF degradation.

Requirement for T4 protein synthesis to inhibit degradation of abnormal proteins

To find whether T4 protein synthesis is needed to block the degradation of *E. coli* protein fragments we used the antibiotic chloramphenicol (CAP) to inhibit protein synthesis at various times after T4 infection. Puromycyl protein fragments in *E. coli* CR63 were labelled with ^{14}C -leucine, as described above; the culture was divided and one half was infected with T4 particles. Our data (Fig. 4) show that T4 protein synthesis is required to inhibit the degradation of ^{14}C -PPF. If CAP is added and T4 protein synthesis is blocked 1 min after the addition of T4 particles, the T4 infected and uninfected cultures show essentially no difference in PPF degradation. CAP does not interfere with T4 adsorption or injection; over 95% of the cells in the 1-min CAP sample had lost their colony-forming ability because of T4 infection. If CAP is added to samples at progressively later times after T4 infection, the samples show progressively more inhibition of PPF degradation. The degradation of PPF when CAP is added 7 min after infection is nearly the same as in the T4-infected sample with no CAP. The addition of CAP 10 min or longer after infection does not affect the inhibition of PPF degradation (data not shown). Therefore, it seems that T4 proteins synthesised early after infection inhibit the degradation of puromycyl peptides. Further, the continued synthesis of these proteins is not necessary to sustain the inhibition for the duration of the latent period.

Maturation-defective T4 mutants¹⁵ have also been used to demonstrate that T4 early proteins are sufficient to inhibit PPF degradation. The proteins encoded by T4 genes 33 and 55 are required continuously for the synthesis of late T4 mRNA; thus, in T4 33⁻ or 55⁻ infections only early proteins are produced.

Our results (Fig. 5) show that in T4 33⁻ or 55⁻ infected *E. coli* cells PPF degradation is inhibited to the same extent as in the control T4 infection. We conclude that inhibition of PPF degradation is an early T4 function.

The extent of T4-induced inhibition

It is difficult to assess with certainty the extent to which T4 inhibits CCP and PPF degradation. The continued solubilisation of labelled proteins that we have observed after T4 infection is at least in part due to (1) the time required for T4 particles to adsorb to bacteria, to inject their DNA, and to effect maximum

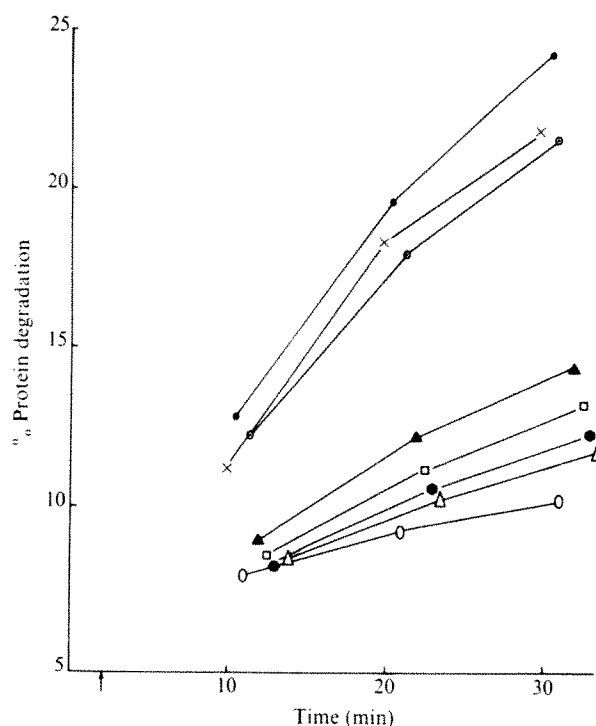


Fig. 4 The requirement for T4 protein synthesis to inhibit degradation of puromycyl protein fragments. This experiment is similar to that described in Fig. 1d, except protein synthesis was blocked at 1 min (○), 4 min (▲), 5 min (□), 6 min (●), and 7 min (△) after T4 infection and immediately after dividing the CR63 culture in one of the uninfected controls (●) by addition of chloramphenicol (CAP) at a final concentration of $100 \mu\text{g ml}^{-1}$. ×, Uninfected control; 0, infected control.

inhibition, (2) the uninterrupted turnover of those normal proteins synthesised along with PPF or CCP during the labelling period, and (3) continued cleavages by peptidases of CCP and PPF molecules that were substantially degraded before T4-induced inhibition became effective. As treatment of cells with organic solvents such as toluene virtually eliminates the degradation of β -galactosidase amber fragments (D. Zipser, personal communication), we were interested to compare the inhibition of PPF degradation observed after treatment with organic solvents to that observed after T4 infection (Fig. 6). The organic solvent used was chloroform, which blocks degradation as rapidly as any treatment we have tested. Our results indicate that addition of chloroform to *E. coli* cells inhibits PPF turnover only slightly more rapidly than does addition of T4 particles (MOI = 5). This difference in kinetics of inhibition is probably due to the longer time required by T4 to maximally block PPF degradation. The turnover rates of PPF in the T4 infected and chloroform treated samples are nearly the same by 10 min after infection.

Inhibition of protein degradation by T5 and T7 phages

Inhibition of degradation of abnormal proteins and protein fragments following virus infection is not peculiar to T4 phages. Other phages, unrelated to T4, such as T5 and T7, also inhibit PPF degradation (Table 1). Neither T5 nor T7 infection, however, seems to inhibit PPF breakdown to as great an extent as does T4 infection.

Implications

We have found that infection by T4, T5, or T7 bacteriophages results in the inhibition of degradation of abnormal *E. coli* proteins (CCP) and puromycyl protein fragments (PPF). Normal *E. coli* proteins, however, continue to turn over at their usual slow rates despite T4 infection. The differential effects of T4 on the turnover rates of normal and of abnormal proteins are not explained by the model of one *E. coli* proteolytic system in which the V_{max} of a crucial degradative enzyme is reduced after phage infection so that at high substrate concentration (for example, in the presence of PPF or CCP) the reduced V_{max} becomes rate limiting, whereas at low substrate concentrations (in the absence of PPF or CCP) substrate is limiting. On the contrary, our experiments (Fig. 2) show that in the same T4-

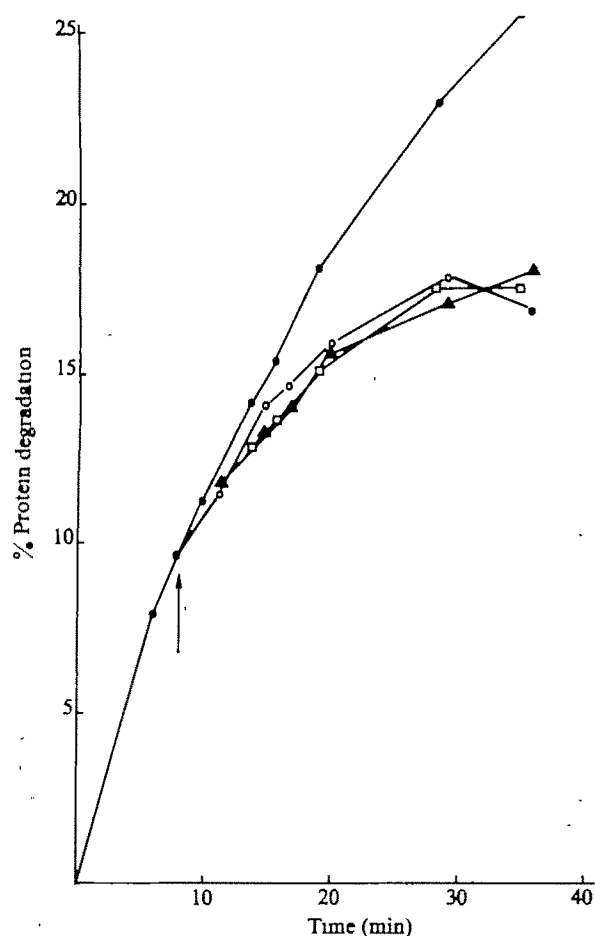


Fig. 5 Inhibition of PPF degradation after infection by T4 maturation defective mutants. This experiment is similar to that described for Fig. 1d with the following exceptions: an *E. coli* B (*Lon*⁻) culture containing ¹⁴C-PPF was divided into four aliquots, one of which was infected with T4am N134 (gene 33⁻) (○), another with T4am BL292 (gene 55⁻) (▲), the third with wild type T4 particles (□), and the fourth was treated only with broth (●). Degradation of ¹⁴C-PPF in each aliquot was assayed as described in Fig. 1.

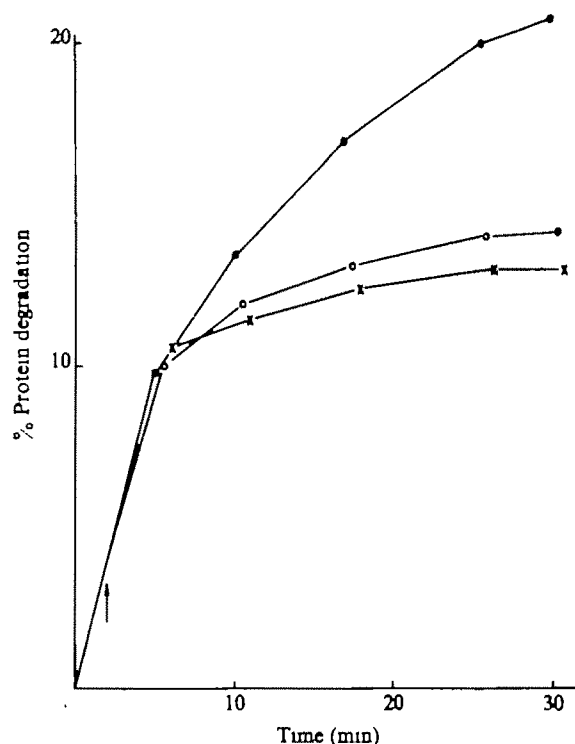


Fig. 6 The effect of chloroform on degradation of PPF. This experiment is similar to that described for Fig. 1d, except that several drops of chloroform were added to one aliquot of the ¹⁴C-PPF-containing *E. coli* CR63 culture. ●, Treatment with broth only; ○, T4 infection only; ×, chloroform added.

infected cells at the same times that CCP (or PPF) degradation is inhibited, the degradation of normal proteins is unaffected. Thus, high substrate concentrations are not related to the differential effects of T4 infection on the degradation of normal and of abnormal proteins. Our results suggest that, at least in part, the *E. coli* pathway for the degradation of abnormal proteins is distinct from the pathways for the degradation of normal cellular proteins. Therefore, the turnover of most normal *E. coli* proteins is not a consequence of changes in these proteins which renders them susceptible to degradation as abnormal proteins.

Our observations explain why nonsense fragments of most T4 proteins are readily found in extracts of infected cells⁶, whereas *E. coli* nonsense fragments are usually not found due to their rapid degradation in uninfected cells. This difference in turnover rates is not the result of some intrinsic distinction between T4 and *E. coli* proteins, but rather of the inhibition of CCP and PPF degradation following T4 infection.

Inhibition of CCP and PPF degradation requires T4 protein synthesis. Adsorption of T4 particles to the *E. coli* surface and injection of T4 DNA are not by themselves sufficient to alter protein turnover. The results of experiments using chloramphenicol to block T4 protein synthesis at various times after infection suggest that an early T4 function is responsible for inhibition of CCP and PPF degradation. This suggestion is consistent with our observations following infections by T4 maturation defective mutants. In T4 33⁻ or T4 55⁻ infection, early proteins, but no late proteins, are made; nevertheless, degradation of CCP and PPF is inhibited to the same extent as in wild type T4 infection. We conclude, therefore, that early T4 protein(s) shut off the *E. coli* system for degradation of abnormal proteins and protein fragments.

The fact that several unrelated phages are able to inhibit CCP and PPF degradation in their host cells suggests that this ability may have some evolutionary significance for the survival of these phages. Perhaps specific proteins of various phages and

other viruses would be degraded as though they were abnormal proteins by the host cell if its degradation system were not inhibited.

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letters to nature

An ultraviolet subdwarf companion to HD17576

ULTRAVIOLET objects detected by the S2/68 UV sky-survey telescope¹ aboard the TD-1 satellite consist primarily of known early-type stars. In addition, there is a substantial subgroup of unidentified and/or untyped ultraviolet stars including some new hot subdwarfs^{2,3} and a small subgroup of known late-type objects including the 20 stars of types F and G listed in the Ultraviolet Bright-Star Spectrophotometric Catalogue⁴. Selection of those objects whose ultraviolet colours suggest that they are earlier than an unreddened B5 V star ought to eliminate the latter category entirely, but several apparently late-type anomalies persist. If these objects cannot be discarded on grounds of blending, misidentification, inaccurate ultraviolet colours (objects with low flux or few orbital passes), or erroneous prior spectral types taken from the literature, then one is confronted with the possibility of genuine duplicity. UV0246-37, taken from the UCL Catalogue of Ultraviolet Objects soon to be published, is just such an object: a binary system consisting of a previously unrecognised hot ultraviolet star and a cooler G-type primary which wholly dominates in the visible region.

Six unblended spectral scans of UV0246-37 were obtained by the S2/68 experiment in 1972-3, two of which must be rejected: in one pass the target lay too far off-centre to yield reliable data, and in the other the data were contaminated by the enhanced radiation background of the South Atlantic Anomaly⁵. Photon pulse counts were obtained in three 400-Å spectrometer channels centred on 1,550 Å (A2), 1,950 Å (A3), and 2,350 Å (A4), and in a photometric channel with FWHM 310 Å centred on 2,740 Å (A1 channel). These counts were converted to flux densities through the absolute calibration determined by Humphries *et al.*⁶ The mean ultraviolet spectrum from the four passes is plotted in Fig. 1, excluding the data at 1,750 Å and 2,150 Å where vignetting was severe. The steep blueward rise is reflected in the mean difference between the magnitudes derived from pulse counts in the A1 and A2 channels: $\overline{m_{A2} - m_{A1}} = -2.00$.

The position of UV0246-37 coincides with that of HD17576 (=DM-37° 1050 = SAO193923), a star of $m_{pg} = 8.1$ classified as G0 on two HD plates⁷. To verify the spectral classification and check for evidence of duplicity, uvby and β photometry was conducted on the 1-m telescope at the South African Astronomical Observatory in July 1977; and spectra at reciprocal dispersion 30 Å mm⁻¹ (second-order blue) were obtained on backed Ila0 plates with the f/1.4 EMI image-tube spectrograph at Cassegrain focus on the 1.9-m telescope in September 1977.

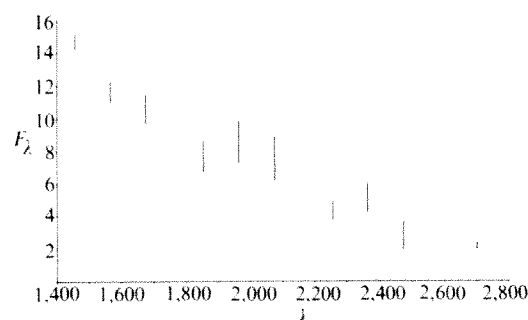
The luminosity class of the G0 star—as predicted among other factors on the strength of the G band, the dominance of Hδ over the Fe I and Sr II lines shortward of it, and measures of luminosity-sensitive line ratios⁸—must be V or IV-V. The only spectroscopic trait indicative of the presence of a hot companion is the ratio of the Ca II lines (K:H ≈ 3:4), long held to be a reliable diagnostic of a composite spectrum⁹ when the 'cool' primary is later than type F and the secondary earlier than type A.

The composite nature of HD17576 is strikingly confirmed by the uvby photometry (for a description of the uvby system, including the passbands of the four filters, see ref. 10):

$$\begin{aligned} V &= 7.82 \pm 0.02; u - b = 1.152 \pm 0.006 \\ b - y &= 0.382 \pm 0.006; m_1 = 0.128 \pm 0.010 \\ c_1 &= 0.113 \pm 0.010; \beta = 2.584 \pm 0.007 \end{aligned}$$

(weighted means of three observations). Since m_1 is low and c_1 very low indeed compared with the approximate mean values (for G0 V) of 0.18 and 0.34 respectively (E. Oblak, personal communication and ref. 11), it stands out conspicuously in a plot of the reddening-insensitive indices [c_1] against [m_1] (Fig. 2). On the other hand, $(b - y) = 0.382$ is not anomalous and though not a reliable luminosity discriminant further supports the G0 V classification of HD17576 since $(b - y)$ remains virtually unaffected by any faint hot companion. [$u - b$] = [c_1] + 2[m_1] = 0.369 is certainly bluer than normal (~0.76); had it not been

Fig. 1 Mean ultraviolet spectrum of UV0246-37 from the S2/68 experiment: nine unvignetted points in the wavelength region covered by the spectrophotometer channels (A2, A3, and A4) and the photometric datum at 2,740 Å (A1 channel). Ordinate units are 10^{-12} erg cm⁻² s⁻¹ Å⁻¹; abscissa units, Å. Error bars indicate the 1σ deviation over four of the six recorded orbital passes (see text).



contaminated, it could well have served as a luminosity discriminant¹¹. The H β measurement is slightly low (Crawford¹² tabulates 2.606 as the norm for G0) but among the G stars the β index loses most of its usefulness as a luminosity or temperature discriminant through the crowding of absorption lines.

The presence of the ultraviolet star in a binary system with a normal late-type primary enables us to infer the absolute luminosity of the hot secondary. The standard luminosity calibration gives $M_v = 4.4$ for a main-sequence G0 star^{13,14}; with $m_v = 7.82$, the distance turns out to be only ~ 50 pc.

On the absolute flux calibration scale determined for Vega by Hayes and Latham¹⁵, the apparent magnitude of UV0246–37 at 2,740 Å is $m_{A1} = 8.07$. To derive m_v for the hot secondary, the UV flux curve can be extrapolated—a possibly reprehensible procedure in view of the 0.2 or 0.3 mag uncertainty incurred by the Balmer jump—to give $(m_v - m_{A1}) = 2.0$. Alternatively, we can use the fact that the flux distribution of UV0853+01, an early-type field star unaffected by the presence of a cool companion, mimics that of UV0246–37 to infer the same 2.0 mag difference. Moreover, if the mean value $\overline{m_v - m_{A1}}$ is calculated for all hot subdwarfs (J. R. Giddings, unpublished data) with ultraviolet colours $(m_{A2} - m_{A1})$ and $(m_{A2} - m_{A4})$ deviating no more than ± 0.2 from those for UV0246–37, we arrive once more at the same result.

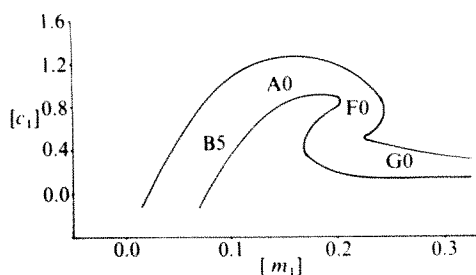


Fig. 2 Boundaries of the main sequence in the $[c_1]$ – $[m_1]$ plane, with normal positions for several spectral types shown. In standard notation¹⁰, $[c_1] \equiv c_1 - 0.20(b - y)$ and $[m_1] \equiv m_1 + 0.18(b - y)$. The diamond represents HD17576, whose anomalous position reflects its composite nature. The main-sequence band is based on the observational material of Oblak *et al.*¹¹.

Thus $m_v \approx 10.1$ for the secondary and its absolute magnitude follows: $M_v = 6.7$. If the determination of MK luminosity were in error by half a class interval, a not uncommon discrepancy¹⁶, then the quoted value could be 0.6 mag too faint. In any case it is at once evident that the secondary is markedly subluminal for an early-type star albeit more luminous than any white dwarf; it is indeed a hot subdwarf akin to such objects in the UCL Catalogue as UV1758+36 (ref. 2), UV0921+36 (BD +37° 1977) (refs 17–19), and UV2158–02 (BD –3°5357) (ref. 20) but far more subluminal. Nevertheless, it is not without precedent: four objects in the list of subdwarf O stars by Greenstein and Sargent²¹ (HZ 38, HZ 3, Feige 26, and BD +25°4655) have $M_v \geq 6.0$. Luyten²² considers the kinematic data for O and B subdwarfs scarcely distinguishable and accordingly treats them as a single group for which, on the basis of his proper-motion survey of faint blue stars, he finds $\overline{M_v} = 4.1$. Evidently UV0246–37 has an exceptionally low luminosity, even for a subdwarf, indeed approaching that of the most luminous white dwarfs. However, the values of M_v for the hottest white dwarfs are ill determined: for HZ 43, assigned $M_v = 7.1$ by Greenstein and Sargent²¹, a careful study by Margon *et al.*²³ finds $M_v = 9.1 \pm 0.5$. Subdwarfs are in any case readily distinguished from white dwarfs on photometric and spectroscopic criteria (for example, ref. 24) except in a special case like the present one where the required observational data cannot be simply ascertained.

At this stage, estimation of the physical parameters of UV0246–37 is an invidious task inasmuch as the errors in the flux measurement are substantial and T_{eff} estimates from a black-body model are notoriously sensitive to the slope (for example, ref. 23). A crude black-body fit yields a temperature of $4.2^{+0.5}_{-0.7} \times 10^4$ K. It may be noted that HZ 38, an O subdwarf with the same luminosity²¹ as the one at hand, has $T_{\text{eff}} = 36,000$ K and $\log g = 7.0$.

The significance of the discovery reported here is twofold. Carnochan *et al.*¹⁷ have mustered evidence from the faint (limiting $m_v \sim 10$), unclassified ultraviolet objects detected by S2/68 that there exists a substantial population of subluminal hot stars in a region of the HR diagram previously considered sparsely populated. Their space density and scale height have been calculated and will be published elsewhere. UV0246–37 is manifestly drawn from this population.

Second, there is a growing body of recognised cool stars with hot secondaries^{20,25,26}, few of which represent such extreme cases of spectral segregation as UV0246–37, the primary being undetectable in the ultraviolet region and the presence of the secondary nearly indiscernible in the visible. The S2/68 survey yielded several other late-type ultraviolet anomalies of which ground-based spectroscopy and photometry are being carried out (ref. 27 and J. P. Swings and M. Berger, personal communications), and it is anticipated that other cool stars will prove to have unsuspected hot companions. These objects are prime candidates for investigation by the International Ultraviolet Explorer satellite since their fortuitous occurrence in binary systems will help to elucidate the nature of the hot subdwarfs.

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The origin of lunar palaeomagnetism

THE new determination of magnetic field anomalies over part of the Moon's surface from Apollo 15 and 16 subsatellite magnetometer data has recently been interpreted¹ in terms of magnetised areas of the lunar surface. I show here that, from this analysis, palaeomagnetic pole positions can be calculated and that these are so clustered on the lunar surface that there is evidence against meteoritic or cometary processes as explanations of the remanent magnetisation of the Apollo rocks. I conclude, therefore, that the Moon had a magnetic field in its early history produced by dynamo processes in a fluid electrically conducting core.

Since the discovery of remanent magnetisation of Apollo 11 lavas², we have attempted in a series of papers to interpret lunar palaeomagnetism as observed in the remanent magnetisation of the returned Apollo crystalline rocks and breccia and in widespread crustal magnetic anomalies mapped by magnetometers on Explorer 35³ and the Apollo 15 and 16 subsatellites⁴, and by the reflected electron technique⁵. Surface traverses of Apollo 14 and 16⁶ also detected magnetic anomalies. Our interpretation supposed that the Moon once had a magnetic field in which these rocks acquired, at the time of their cooling from above the Curie point of their iron grains, a stable thermoremanent magnetisation. This field existed at least from 4,000 to 3,200 Myr (the ages of the rocks studied), decreased from 1G to 5,000 nT (ref. 7) over this interval and has now disappeared. Much scepticism has been expressed⁸ about this hypothesis of an ancient internal field, presumably because a number of consequences follow which had not been envisaged by the majority of lunar scientists—the existence of a sizeable iron core in which a dynamo process can take place⁹, the melting of the entire Moon¹⁰ in its earliest history (so that the iron core formed) and thermal convection in the interior.

Consequently there have been several suggestions as to how the magnetisation of the lunar rocks might have been acquired by an external field¹⁰ (or no field at all, although as T. Gold points out, some far-reaching modification in physics, for example, the law of parity, would seem to be necessary if this explanation were to be sustained). These ideas appeal to processes with which we are unfamiliar in terrestrial studies, and they are therefore difficult to test. For example, the ubiquity of large meteorite impacts on the early lunar crust has suggested the possibility of impact magnetisation^{12,13}. Also it has been pointed out that cometary impacts are statistically likely to have been quite frequent on the Moon and again the physical processes during such events may have favoured magnetisation¹⁴. What must be postulated is that the acquisition of a stable remanent magnetisation is greatly facilitated during such large impacts either by the effect of the transient stress field on the

iron grains (although the magnetisation direction might be biased by the stress) or through the shock wave raising the rock temperatures instantaneously to above the blocking temperature of the iron grains. Thus the magnetising field on such a hypothesis could be small and transient: it may arise from surges of electric current in the plasma during impact, 'pseudo-lightning strikes', or be the field of the solar wind, which does not remain constant for long. We are concerned with the early history of the Solar System when the solar wind field may have been much more intense than at present. The average solar wind field now lies in the ecliptic, therefore a magnetisation of the Moon along its rotation axis is implausible if the magnetisation was acquired very quickly as must be the case in an impact magnetisation process¹⁵. On the other hand, because of the Moon's rotation, a magnetisation in the ecliptic or the lunar equatorial plane is unlikely unless the magnetisation was acquired in a time much shorter than a month.

Very important interpretations of the lunar magnetic anomalies, as seen by the Apollo 15 and 16 subsatellites, which bear on these critical questions, have been made by Hood, Coleman and Russell¹. They show that these magnetic anomalies at altitudes of 100 km can be represented by 35 dipoles at the surface of the Moon and they calculate the strength and direction of these dipoles. Strangway¹⁶, and Dermott and Runcorn (unpublished) found that the largest magnetic anomalies of the lunar far side demand east-west directed dipoles for their explanation. Hood *et al.* show that the dipoles are not randomly directed in space but lie nearly in the equatorial plane of the Moon. However, they show that their directions, projected in this plane, are scattered, apparently randomly, through 180°. They conclude that this random feature is the explanation of the null result found in the determination of the present dipole moment of the Moon¹⁷. This result I had explained¹⁸ by showing that a spherical shell permanently magnetised by a field of internal origin, so that the magnetisation is parallel and proportional to the field, which later disappears, produces no external field. I had argued that such magnetic anomalies would arise from the large craters releasing lines of magnetic force from the magnetised lunar shell. Thus the anomalies should be well modelled by a dipole in the centre of the crater but it has been often found in such attempts (ref. 1 and Dermott and Runcorn, unpublished) that the dipoles are not so positioned, although the largest magnetic anomalies are associated with the large craters around Van de Graaf on the far side. It therefore seems likely that the magnetised areas may sometimes result from the cooling of layers of breccia thrown out by meteoritic impacts, as suggested by Strangway *et al.*¹⁶.

The two features of the distribution of dipole directions, that they are near to the lunar equator and in that plane are randomly directed, seem to suggest that a field is responsible which is directed in this plane and this suggests the solar wind field which, though fluctuating, is on average in the plane of the ecliptic, to which the lunar equator is inclined by only 1°. Cometary or

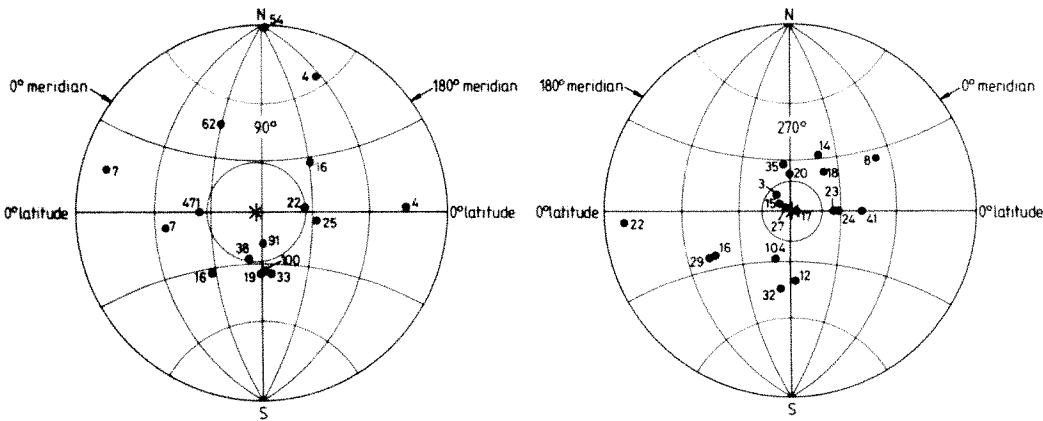


Fig. 1 Distribution of pole positions from lunar magnetic anomaly dipoles (given in Table 1 of ref. 1). Stars are mean directions and circles the 95% circles of confidence. Stereographic projection of eastern (a) and western (b) hemispheres of Moon. The numbers are the dipole strengths per unit area given in G cm. Left, eastern hemisphere. Right, western hemisphere.

Table 1 Number of poles within 30° sectors of longitude compared with number expected on the theory that the dipoles are randomly orientated in the lunar equatorial plane

Longitude sectors	No. of poles all included		No. of poles magnitudes <10 G cm excluded	
	Observed	Theory	Observed	Theory
Eastern Hemisphere				
0–30°, 150°–180°	2	3.1	0	2.3
30°–60°, 120°–150°	6	3.7	4	2.7
60°–90°, 90°–120°	7	6.0	7	5.9
Western Hemisphere				
180°–210°, 330°–360°	1	3.8	1	3.6
210°–240°, 300°–330°	4	4.5	4	4.2
240°–270°, 270°–300°	13	9.6	12	9.1

$\chi^2 = 5.30$ (all poles included) and 5.95 (excluding <10 G cm). Confidence limits for rejection of theory, 75% and 80% respectively.

meteoritic impact hypotheses seem to be supported. However, Table 1 of Hood *et al.*¹ gives the locations on the Moon of these dipoles and their angles of orientation to the vertical and the meridian are listed. I have calculated the corresponding pole positions, as has proved fruitful in palaeomagnetism, that is, the latitudes and longitudes of the axes of those dipoles at the centre of the Moon which have fields at the various locations coincident with the palaeofield directions¹⁹ found there. These poles are plotted in Fig. 1 and are seen not to be random. A minority of directions are opposed to the most frequent grouping: this is to be expected as a dipole may either arise from a magnetised body of rock or from a magnetised part of the lunar crust in which there is a demagnetised volume, a physical cavity formed by impact or a volume of rock demagnetised by impact. Moreover, reversals of polarity of a field produced by a core dynamo process is to be expected. The scatter of the pole positions is considerable but this is to be expected as many of the anomalies, so remarkably analysed and modelled by Hood *et al.* are much less than 1 nT, and their treatment of the magnetometer data has required the removal of the much larger field of the Earth's magnetotail, which is not very constant. In fact the nonrandom distribution of the pole positions demonstrates that this difficult process has been successfully accomplished by Hood *et al.* and suggests that an attempt to separate the magnetic anomalies from other regions of the Moon, at present obscured by the solar wind field, might be worth attempting.

Thus the demonstration that the vector means of the two groups of pole positions, shown with their 95% circles of confidence in Fig. 1, are at 180°, seems at first sight to be strong evidence that the magnetisation of the lunar crust does not arise from meteoritic or cometary impacts, for the palaeomagnetic directions impressed on the lunar crust from such processes must, because of the lunar rotation, be instantaneous directions from the solar wind magnetic field. Unless all the impacts occurred at the same time of the month, the palaeomagnetic directions over the Moon as a whole would not have an axis fixed in one region of the Moon. However, the fact that the mean poles are along the E–W equatorial axis is odd. Consider a set of dipoles at a site (0° N, 180° E) lying in the equatorial plane. If the difference in azimuths of two neighbouring dipoles is dI then their poles lie on the equator at distance $d\theta$ apart where θ is the angular distance from the site

$$d\theta = dI(1 + 3 \cos^2 \theta)/2$$

Thus if these dipoles at one site are distributed randomly through 180°, then their poles are clustered four times more densely about an E–W equatorial axis than about the N–S axis. If random N–S components are added, this distribution would resemble that of Fig. 1. However, I show now that the conclusion that the dipoles are random is wrong, although clearly the dipole

directions will be substantially affected by random components inherent in the magnetic survey observations and analyses. It is significant that four out of six of those poles which lie far away from the main group have dipole moment per unit area magnitudes less than 10 G cm, that is corresponding to fields less than 0.1 nT at the subsatellite orbit.

The sites of the dipoles are in fact distributed over a strip 45° on either side of the mean site at 10° S 180° E. If random dipoles were uniformly distributed over the equator between 135° E and 215° E then the pole density distribution would be proportional to $\tan^{-1} \sec 2\theta$, with a maximum at 90° E and 270° E, three times that of the minimum at 0° and 180°.

On the above hypothesis the proportion of poles within the 30° ranges of longitudes is shown in Table 1 and is compared with the actual number. It is clear that χ^2 test excludes at the 80% confidence level the hypothesis that the directions are wholly random: an east–west component is also present.

Yet there is an external field to which the Moon is exposed during 2–3 days around full Moon and which is directed along the Earth–Moon line. This is the Earth's magnetotail: the Moon traverses both its north and south lobes, in which the magnetic field is directed towards and away from the Sun respectively, in the course of the year. Thus if impacts had imparted to crustal regions magnetisations directed along this field, a plot resembling Fig. 1 would be found. However, the Moon spends 10 times as long in the solar wind as in the magnetotail, and so unless impacts occurred always at full Moon the crustal magnetisations should be nearly randomly distributed in the ecliptic. This hypothesis would, if 90° of rotation of the Moon about its axis since magnetisation had occurred, explain the results of Table 1, had the magnetised area been small. But for two areas of longitude difference $d\lambda$ within the strip, imprinted by the magnetotail field, their calculated pole positions would be $d\theta$ apart where $d\theta = 3d\lambda(2 + \tan^2 \lambda)/(4 + \tan^2 \lambda)$. This pole distribution is spread nearly uniformly over about 70° on either side of the mean pole. Thus the additional hypothesis of magnetisation by the magnetotail field, as well as the solar wind field, would broaden the pole distribution of the theory columns of Table 1, rather than reduce it as required by the observations.

So in spite of random scatter and the restricted area covered by the anomalies, it is concluded that the ancient pole position was not near the present axis of rotation of the Moon. Yet, in spite of the lunar core being an order of magnitude smaller than the Earth's core and the angular rotation less by a factor of 30, the reason originally advanced to explain the fact that the average geomagnetic field is directed along the axis of the Earth's rotation, that is the Coriolis force is many orders of magnitude greater than any other term in the hydrodynamic equation²⁰, must also apply to the Moon. Therefore, I conclude that lunar palaeomagnetism shows that polar wandering occurred in the Moon's early history, that is, the Moon rotated relative to its axis of rotation. It was shown previously that polar wandering can take place in a planet either by internal motion²¹ or by the disequilibrium produced by a mountain²² (or crater), provided that solid state creep in the interior can readjust the position of the equatorial bulge which otherwise strongly stabilises the rotation axis. The dates of the magnetised regions on the lunar highlands, which we are discussing, are very early²³: if the rocks are impact breccias they may be the same age as the last great impacts on the Moon (4.1×10^9 yr)²⁵ or, if they arise from craters in the already magnetised highlands crust, they may be older. In any case, they are of such an age that the great impacts which redistributed mass on the lunar surface on a large scale, were a likely cause of the polar wandering required to explain this result. Further studies when a complete magnetic anomaly map is obtained by the lunar polar orbiter may show pole positions distributed between the present equator and the present rotation pole. Such a polar wandering path would be of great interest, and would perhaps be useful for the relative dating of the lunar far side. Such a slow polar wandering through over 90° would suggest that solid state creep in the lunar interior has played an important part in its evolution and supports the

view that convection is responsible for its non hydrostatic shape, as I have maintained^{23,26}. The axis of minimum moment of inertia is aligned by the Earth so that the 'bulge' is earthward: thus if polar wandering has occurred it may have taken place along the present limb so that the ancient axis is now east-west.

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Short-term storage and wind power availability

IN a discussion on the economics of alternative energy sources, Ryle¹ suggested that wind power in conjunction with short-term thermal storage can provide a viable and attractive source of energy in the UK. Leicester, Newman and Wright² have recently analysed wind data and empirically derived heating demand over a six-month period. On the basis of these data they suggest that short-term (~150 h) storage is inadequate to provide a reasonably reliable supply from wind generators for heating purposes. This conclusion has been criticised by Diesendorf and Westcott³ and, in view of the limited data sample used it is clear that a more extensive analysis is required. Here we use hourly wind and temperature measurements over a 17-yr period (data obtained from the Meteorological Office, Bracknell) to investigate the performance of a simple system in which wind power is used in conjunction with 150-h thermal storage to provide domestic space heating. The results are promising, even for this basic system, and confirm that, with some modifications, such a system can provide adequate reliability. A significant result of this analysis has been to indicate the importance of selecting an appropriate value for the wind speed at which the wind turbine is to produce its peak output (the 'rated' wind speed). It is shown that the use of too high a rated speed not only greatly exaggerates the fluctuations in available power but could also result in a more expensive design of turbine for a given annual energy output.

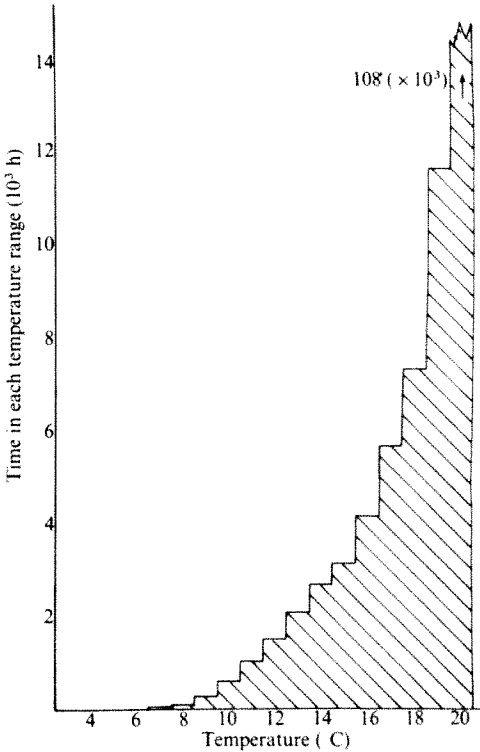


Fig. 1 Histogram of the number of hours spent at different internal temperatures for a 17-yr period (149 × 10³ h).

For the main analysis a model system has been constructed in which electrically connected turbines at three typical well-spaced sites (Aberporth, Kinloss and Manston—three of the eight sites considered by Leicester *et al.*²) are used to provide the sole means of heating three groups of houses, one at each site. Each house is equipped with a thermal store having a half-power decay time of 150 h and the heat loss from the house is taken to be proportional to the difference between internal and external temperatures. (Although heat losses also depend to some extent on cooling by wind and rain, these effects are small and have not been included.) For this simple system the rate of supply of heat to the houses is determined by the natural heat loss from the store, although in a practical system some additional control is likely to be included; the houses themselves have a thermal time-constant of 10 h.

Each turbine has a power rating such that the total energy produced over the winter months (October–March) is just sufficient to maintain the houses at that site at a constant internal temperature of 20°C. Because in the short term wind power and heat losses (that is, external temperature) are uncorrelated, there will be some periods when insufficient power is available so that the house temperature will fall and, conversely, others when the supply of heat from the store is excessive and some has to be discarded. To simulate a national grid system the power from the three turbines is divided in such a way as to keep the internal temperatures of the three groups of houses equal and as close to the design value (20°C) as possible.

Table 1 Adopted turbine characteristics^{4,5}

Wind speed	
Rated wind speed	Relative power
0-0.5	0
0.6	0.2
0.75	0.5
0.9	0.8
1.0-2.0	1.0
>2.0	0

The results of this analysis are shown in Fig. 1 as a histogram of the number of hours spent at different internal temperatures. It is found that the temperature is within 3°C of the nominal value (that is >17°C) for 86% of the time and falls below 10°C for a total of only 462 h in 17 yr (0.3%). It is interesting to examine the results of our analysis for 1 February to 31 March 1975, a period considered as an 'extreme' example by Leicester *et al.*² The histogram for this period is shown in Fig. 2 (solid line) from which it can be seen that, even over this period, the temperature is above 16°C for over half of the time and never falls below 11°C. (Note that, with no change in the results, the system could also provide domestic hot water at a nominal temperature of, say, 60°C, with corresponding temperature fluctuations.) On the basis of these figures the reliability of the system would not seem to be very much worse than that of the conventional grid when the effects of miners' strikes, 3-day working weeks and so on are included.

The system considered here is extremely simple and possible enhancements which would undoubtedly improve the performance still further are: (1) the provision of a limited amount

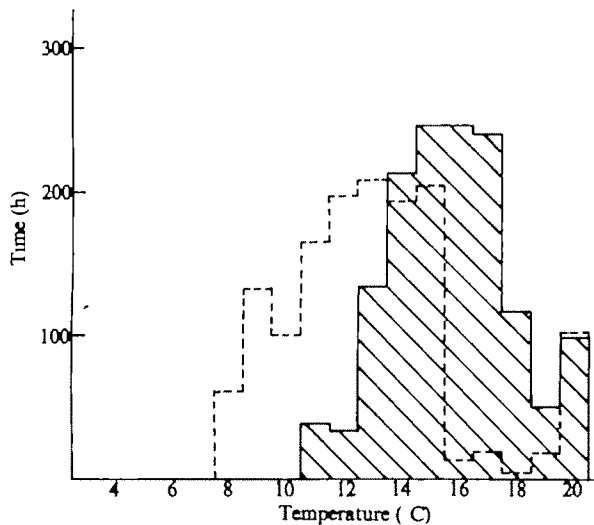


Fig. 2 Histogram corresponding to Fig. 1 for the 'extreme period', February–March 1975 (Leicester *et al.*²) $V_R = 1.5 \bar{V}$ (solid line), $V_R = 2.3 \bar{V}$ (dotted line).

of non-degrading storage, for example, of the eutectic or chemical type which, in conjunction with the above system, would reduce the wastage of energy during warm periods. (2) The use of storage having the same capacity but better thermal insulation (as might be applicable to a small-scale district storage system) so that some control could be exercised over the rate at which heat was extracted. Avoiding the unnecessary heat loss at night, for example, would be important when considering the economics of the system.

We have used the measured performance figures of actual machines^{4,5} and, as described below, adopted a 'rated' wind speed of 1.5 times the mean wind speed, $\bar{V}$. By comparison, the generator characteristics used by Leicester *et al.*² are based on a higher rated wind speed and, also, give an output which seems to fall off unreasonably rapidly at the lower wind speeds. To show the effect of a higher rated speed we have recomputed the histogram of Fig. 2 for a rated speed of $2.3 \bar{V}$ (dotted line) from which it is apparent that the performance of the system is significantly degraded. In contrast to the results of Leicester *et al.*², who assumed an unrestricted rate of energy extraction from the store, in neither case is the store completely emptied over this period. The discrepancy between our results and those of Leicester *et al.*² can therefore be explained largely in terms of (1) the different wind-turbine characteristics which we have used

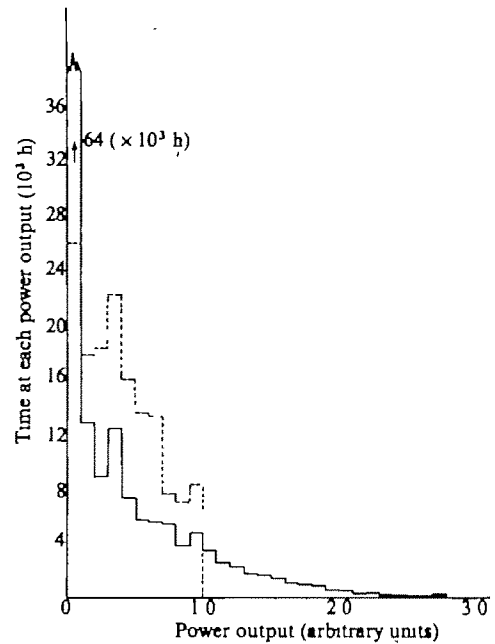


Fig. 3 Temporal distribution of combined power output from the three sites for two values of 'rated' wind speed; $V_R = 2.3 \bar{V}$ (solid line); $V_R = 1.5 \bar{V}$ (dotted line).

(Table 1) and (2) our use of a thermal store with an exponential decay. (However, it is not clear why the averaged heating demand as derived by Leicester *et al.*² should have such a low standard deviation (4.9%), a value which is remarkably small even allowing for a significant, and constant, demand for domestic hot water; in contrast, the weekly records of the energy used for heating our own laboratory between October 1977 and March 1978 show a s.d. of 30%.)

Finally, we discuss briefly the effect of variations in the rated wind speed of the turbine. ETSU⁶ have shown that for UK sites the maximum annual energy output is provided by a turbine having a rated wind speed approximately 2.3 times the mean wind speed, assuming that the cut-in speed, that is the speed below which no output is obtained, is about half the rated wind speed. However, such a turbine gives rise to prolonged gaps in the energy supply. If a lower rated wind speed is adopted these gaps are considerably reduced; conversely, if the rated speed is higher than $2.3 \bar{V}$, not only are the gaps seriously increased but the total energy output also falls. The effect of changes in the rated speed can be seen clearly in Fig. 3 which shows the number of hours for which a given combined output is obtained from the three sites considered previously. The histograms have been derived from two systems having the same total energy output

Table 2 Parameters of two wind turbines having the same annual output but different rated speeds

Parameters	$V_R = 2.3 \bar{V}$	$V_R = 1.5 \bar{V}$
Power rating	1 MW	300 kW
Rated wind speed	27 knots	18 knots
Diameter	46 m	52 m
Relative axial force	1.0	0.54
Relative torque	1.0	0.62
Estimated costs (£10 ³)		
Tower, foundations	22	17
Orientation	4	4
Rotor	30	34
Mechanical transmission	26	16
Alternator	12	5
Other items	14	14
Total cost	£108(000)	£90(000)

but with different rated speeds of 2.3 $\bar{V}$ (full line) and 1.5 $\bar{V}$ (dotted line). It is apparent that the former system has much longer periods of low or zero output and a correspondingly shorter time at which it is near its rated output.

Because, for a given swept area, reducing the rated speed from 2.3 $\bar{V}$ to 1.5 $\bar{V}$ decreases the annual energy output by ~20%, a proportionate increase has to be made to the size of the turbine blades to obtain the same mean output. The latter machine is, nevertheless, likely to be cheaper, because of the reduced ratings of the alternator and gearbox and the lower loading of the tower, as well as providing a more steady output. This is shown in Table 2 in which two designs having the same annual output are compared. The relative costs have been estimated using figures in the ETSU report⁶.

Our analysis has been concerned only with the time variations of available wind power in relation to fluctuations in the demand for space heating. Because of the normalisation carried out at the start of the analysis no knowledge of the absolute power demand is required and the results are independent of the type or number of houses to be supplied by the wind turbine or the precise temperature at which they are maintained. However, an approximate estimate of absolute energy requirements can readily be made. Assuming an average winter heating demand of 2 kW, a typical figure for a medium-sized, well-insulated house, a single turbine of the type considered in Table 2 would be sufficient to maintain about 90 houses at the temperature levels given in Fig. 1.

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New materials in the
Si-C-Al-O-N and related systems

THE concurrent discovery at the Universities of Utah and Newcastle-upon-Tyne of a continuous series of solid solutions, with the 2H wurtzite-type structure, between silicon carbide (SiC) and aluminium nitride (AlN) is reported here. This, together with observations of the additional solubility of aluminium oxycarbide (Al₂OC), suggests a large extension to the already wide field of sialons. It offers the prospect of many more materials in the Si-C-Al-O-N and related systems that are of scientific and technological interest not only as engineering ceramics but also for their physical, chemical, and electrical properties.

Silicon nitride and silicon carbide are leading contenders for gas turbine and other high-temperature engineering applications. Extensive solid solution based on β -Si₃N₄ was first shown by Oyama¹ and by K.H.J. and Wilson² but only a few systems, such as Si₃N₄-Al₃O₃N, Si₃N₄-MgAl₂O₄ and Si₃N₄-

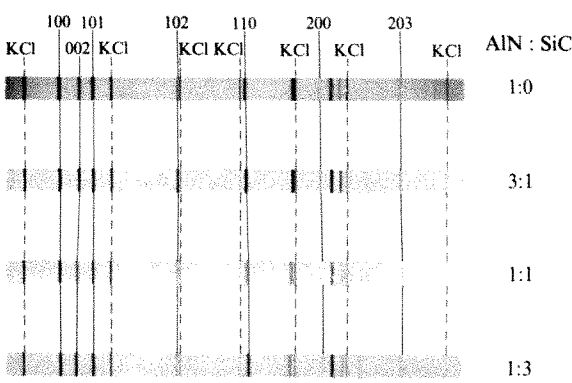


Fig. 1 X-ray photographs of 2H wurtzite-type solid solutions of SiC-AlN.

LiAl₅O₈, have been explored in detail. Investigation progressed in different directions³ when it was appreciated that the reversible replacement



is of general applicability and that the sialons are essentially aluminosilicates in which oxygen is partly replaced by nitrogen. A wide field of new materials exists, vitreous as well as crystalline, built up of (Si,Al)(O,N)₄ tetrahedra in the same way that the almost infinite variety of mineral silicates is based upon the (Si,Al)O₄ tetrahedron.

The formation of extensive solid solutions with silicon nitride and also with silicon carbide might seem surprising in that their covalent bonding, in addition to its high strength, is specific and selective. In the Si-Al-O-N system³ the extended homogeneity range of phases along lines of constant metal:nonmetal atom ratio M:X and the very restricted solubility in directions parallel to the AlN-SiO₂ join is due, at least in part, to the similarity of the two bond lengths

Si-N(1.75 Å) ~ Al-O(1.75 Å) (2)

and the difference between the other two bonds

Al-N(1.87 Å) ≠ Si-O(1.62 Å) (3)

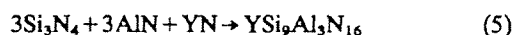
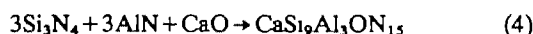
The ready replacement of metal and nonmetal atoms in covalently bonded solids is not restricted, however, to that of Si-N by Al-O. Thus, a variety of polytypes M_mX_{m+1}, M_{m+1}X_m and MX with extended regions of homogeneity have been characterised recently⁴ in the Mg-Si-Al-O-N, Be-Si-Al-O-N and Al-C-O-N systems. Their structures are related to those of the silicon carbide polytypes and are based on the atomic arrangement of wurtzite.

Recent work at Newcastle⁵ has shown the existence of α' -sialons, that is, phases in the M-Si-Al-O-N system where M includes Li, Mg, Ca and Y, that are isostructural with α -Si₃N₄. The unit-cell contents are represented by M_x(Si,Al)₁₂(O,N)₁₆ where $x \geq 2$ and where the metal atoms M occupy partially or completely the only two interstitial sites in the structure. As

Table 1 Unit-cell dimensions of the end members of wurtzite-type solid solutions

	Dimensions (Å)	
	a	c
α -SiC(2H)	3.076	5.048
AlN	3.114	4.986
Al ₂ OC	3.19	5.09

shown by equations (4) and (5), the formation of α' -sialons from silicon nitride



is essentially by replacement of Si-N by Al-N and not by Al-O as in β' formation.

Although the preparation⁶ of wurtzite-type Al_3CON shows the possibility of producing carbon-containing sialons, the first attempts to do so at Newcastle were unsuccessful. Because diffusion coefficients in covalent solids are extremely small, solid solution is unlikely to be obtained by the heating and annealing of the powdered solid components; nor is it possible by melting. The other possibilities, by liquid-phase and vapour-phase reactions, have both now been used in preparing SiC-AlN solid solutions.

The preparation of silicon carbide and silicon nitride from silica by reduction with carbon or carbon plus nitrogen is a vapour-phase process⁷⁻⁹ involving the formation of SiO and CO. Similar reactions occur in the production of aluminium nitride from aluminium oxide (I.B.C., unpublished) and of sialons from clay¹⁰ and $\text{SiO}_2 : \text{Al}_2\text{O}_3$ gels (A. Hendry and K.H.J., unpublished). All such processes occur rapidly at relatively low temperatures, 1,400–1,600 °C. By using a very finely divided, high surface area, amorphous silica ('Cabosil') and a carbon source such as starch or sugar, an intimate and porous mixture of carbon and silica is produced by coking to remove all volatiles. Other oxides may then be homogeneously dispersed within the mixture by precipitation from their soluble salts. Using these techniques, with aluminium precipitated as hydroxide, reactions have been carried out at Utah in both argon and nitrogen atmospheres at 1,600 °C. These show 2H wurtzite-type solid solutions between SiC and AlN and between SiC and $\text{Al}_2\text{O}_3\text{C}$ extending in each case from 2m/o to 100m/o of the aluminium-containing component. In each case continuous shifts of reflections were observed in a single-phase wurtzite-type

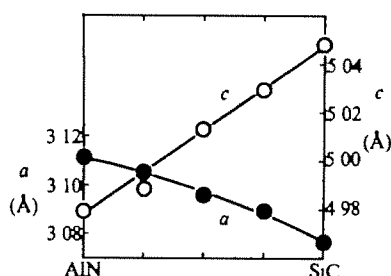


Fig. 2 Unit-cell dimensions of 2H solid solutions of SiC-AlN.

diffraction pattern corresponding with the unit-cell dimensions for the end members given in Table 1.

At Newcastle it was considered that α' -sialons were obtained by reaction of Si_3N_4 with AlN plus relatively small concentrations of CaO or Ca_3N_2 because of the formation of a liquid phase in which the reactants have some solubility. When α' - $\text{CaSi}_2\text{Al}_3\text{ON}_{15}$ was reduced in a carbon crucible at 1,800 °C for 30 min in flowing nitrogen the product was single phase with a wurtzite-type structure of unit-cell dimensions corresponding to 3SiC. AlN. No calcium was detected in the product and in the same experimental conditions calcium oxide was completely reduced and volatilised as calcium vapour and carbon monoxide. In subsequent experiments at the same temperature, mixtures of Si_3N_4 , AlN and CaO were reacted with carbon instead of using the preformed α' -sialon. It is thought that the addition of CaO provides a transient oxynitride liquid in which the reactants are soluble.

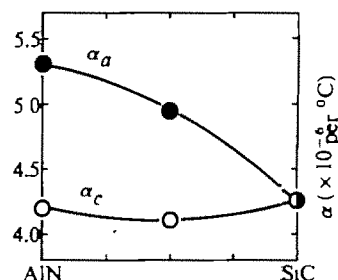


Fig. 3 Linear coefficients of expansion (20–1,000 °C) of solid solutions of SiC-AlN.

SiC-AlN solid solutions were also obtained at Newcastle by heating intimate mixtures in molecular nitrogen at 1,400–1,500 °C of SiO_2 , Al_2O_3 and C obtained by a sol-gel process. The products always contained some β -SiC, unlike those prepared by essentially the same process at Utah. The formation of β -SiC depends on impurities, and also on nitrogen pressure, but it is clear from the present results that β , once formed, does not readily react with AlN to give a solid solution.

Figure 1 shows X-ray photographs of 2H solid solutions of 3AlN:SiC, AlN:SiC and AlN:3SiC compared with that of pure AlN. The shifts of X-ray reflections show that c increases and a decreases as AlN is replaced by SiC. This variation in cell dimensions is shown by Fig. 2. The mean coefficients of expansion α_a and α_c at 20–1,000 °C for the composition AlN:SiC are compared with values for the end members in Fig. 3. The value of about 5×10^{-6} per °C is probably too high for the use of the material in gas turbine applications but the electrical and semi-conducting properties are expected to be of interest.

As extensive solubility between the end members of the SiC-AlN solid solution occurs with $\text{Al}_2\text{O}_3\text{C}$, five component Si-C-Al-O-N phases must occur although their ranges of homogeneity have not yet been established. Moreover, there are many nitrides, oxynitrides and carbo-oxynitrides with wurtzite-type structures, for example LiSi_2N_3 , MgSiN_2 , LiSiON , MgSiAlN_3 and Al_3CON , and so in appropriate conditions these should also form solid solutions with SiC-AlN.

The new materials based on Si-C-Al-O-N in combination with a variety of network-modifying metals will not all be useful ceramics but their other properties are worth investigation. Moreover, they provide a new and hitherto unexplored area of inorganic chemistry.

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New isotopic evidence for the origin of Red Sea brines

THE hot brines in the Atlantis II Deep in the Red Sea (Fig. 1) have concentrations of both oxygen- and hydrogen-isotopes which are different from those of the overlying Red Sea deep water but are identical to the surface waters, only at the southern parts of the Red Sea^{1,2}. It was inferred, therefore, that these brines originated in the southern Red Sea and have migrated northwards to their present location. This hypothesis has often been questioned, using mainly geological arguments, the most striking of which is the improbability of a plumbing system which had to work over a distance of more than 800 km (refs 3, 4). However, the geochemical problem of the depletion in heavy isotopes in the hot brines as compared to the directly overlying seawater has still not been solved. We can add new data to this discussion from stable isotope analyses carried out on new brines which were found throughout the Red Sea (Fig. 1). Many of these occurrences are between the Atlantis II Deep and the southern Red Sea⁵.

Our analytical procedures were the same as those for the early isotope analyses on the Atlantis II Deep brines¹ and are described in detail elsewhere^{6,13}. The accuracies were $\pm 0.07\%$ and $\pm 0.5\%$ for oxygen and hydrogen, respectively.

The results are given in Table 1 and Fig. 2. Many of the brines have chlorinities which are appreciably lower than the nearly saturated brine in the Atlantis II Deep. We assume that this is due to dilution by seawater after intrusion of the saturated brines. This would have caused a change in the isotopic compositions if seawater and brines differed in their initial isotope content. Therefore, we calculated the assumed compositions of the brines before their dilution. These 'corrected' values are shown in Table 1 and Fig. 2.

The brines exhibit a considerable variation in their isotopic content. However, those brines near the Atlantis II Deep are isotopically identical. These include the Atlantis II Deep itself, the Discovery Deep, the Chain Deeps and the Albatross Deep (Fig. 1). This homogeneity supports previous assumptions^{5,7} that the brines in the Atlantis II Deep area are from one source and

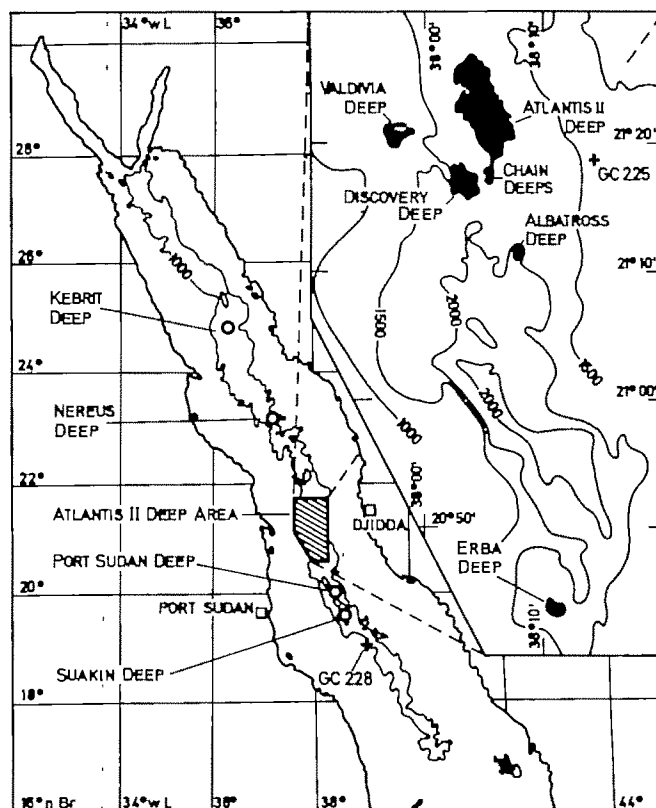


Fig. 1 Locations of Atlantis II Deep brine pools and of new brine occurrences in the Red Sea^{5,19}.

probably have subsurface connection. The presence of nearly identical brines in the Albatross Deep is now understood better and could not be explained by the overspill theory⁸.

The Valdivia Deep is an elevated position located away from the central rift 10 km west of the Atlantis II Deep system (Fig. 1). Its brines have significantly higher D/H and $^{18}\text{O}/^{16}\text{O}$ ratios than the Atlantis II Deep brines. They are the only example of brines which are isotopically identical to present-day Red Sea water, which suggests that they originated from present-day Red Sea deep water, or from Red Sea palaeowaters of present-day composition. For this brine pool, a dissolution of exposed evaporites⁹ by present day Red Sea deep waters is likely.

The brines from the Erba, Port Sudan and Nereus Deeps are enriched in both D and ^{18}O and exceed the range of present-day Red Sea deep waters. Only a few processes could have caused a simultaneous enrichment of D and ^{18}O as shown in Fig. 2 for these three brines. An enrichment by evaporation is highly unlikely as these brines have never been exposed to air. Mixing of recent Red Sea water with only one subsurface brine having a specific isotopic composition is excluded as the adjustment of these values to brines unmixing with seawater (corrected values in Table 1) should then result in identical isotopic compositions, and the corrected values differ significantly. The δD and $\delta^{18}\text{O}$ values of the three brines under discussion fall nearly on a line in the $\delta\text{D}/\delta^{18}\text{O}$ diagram; this line is located between present-day Red Sea deep water and Red Sea deep water of the last glacial maximum 18,000 yr BP (III in Fig. 2). This value was estimated using ^{18}O -variations in foraminifera in Red Sea sediments which are given in Fig. 3. The total ^{18}O -enrichment, $\Delta^{18}\text{O}$, during the last glacial maximum was $\sim +2\%$ in ^{18}O as compared with present-day values. The $\Delta^{18}\text{O}$ values in Fig. 3 are the approximate ^{18}O -oscillations of the Red Sea waters during the past 20,000 yr as surface water temperatures did not change significantly during this period¹⁰. The Red Sea deep water-values given in Fig. 2 have been estimated with the assumption that the $\delta^{18}\text{O}/\delta\text{D}$ relationship of Red Sea deep water relative to open ocean water (SMOW) (that is $\delta\text{D} = 6\delta^{18}\text{O}$ (ref. 1)) has prevailed.

Table 1 $^{18}\text{O}/^{16}\text{O}$, D/H-isotope and chloride data for waters of various brines in the Red Sea

Location	Cl (gper kg)	$\delta^{18}\text{O}^*$ (‰)	δD^* (‰)	$\delta^{18}\text{O}^\dagger$ (‰)	$\delta\text{D}^\dagger$ (‰)
Atlantis II Deep	+156.0	+1.21	+7.4	+1.19	+7.3
Chain A-Deep	+154.8	+1.29	+8.2	+1.26	+8.0
Chain B-Deep	+155.5	+1.19	+7.5	+1.16	+7.3
Chain C-Deep	+154.2	+1.22	+7.5	+1.19	+7.3
Discovery Deep	+155.1	+1.16	+7.5	+1.13	+7.3
Albatross Deep	+143.5	+1.20	+7.7	+1.10	+7.2
Valdivia Deep	+144.6	+1.84	+11.5	+1.83	+11.5
Erba-Deep	+83.8	+1.98	+13.0	+2.09	+14.9
Port Sudan-Deep	+124.8	+2.39	+15.0	+2.57	+16.2
Nereus-Deep	+129.5	+2.64	+17.2	+2.86	+18.9
Kebrit-Deep	+153.2	+1.05	+10.4	+1.00	+10.3
Suakin-Deep	+85.8	+2.56	+21.6	+3.35	+33.6

δ -values are reported versus SMOW and are defined as

$$\delta = \frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \times 1,000 (\text{‰})$$

where R is the D/H and the $^{18}\text{O}/^{16}\text{O}$ ratio respectively.

* Measured values. The original brines are supposed to be diluted by Red Sea Water so the corrected data for undiluted brines are calculated according to the following formula:

$$\delta_{\text{corr}} = (\delta_{\text{Brine}} - x_1 \delta_{\text{SW}}) / (1 - x_1)$$

δ_{Brine} are the measured isotope data*, δ_{SW} the data of recent Red Sea deep water ($\delta^{18}\text{O}_{\text{SW}} = 1.89\text{‰}$, $\delta\text{D}_{\text{SW}} = 11.5\text{‰}$ (ref. 6)). The sea water contributions x_1 to the actual brines are calculated from the respective contents $\text{Cl}^-_{\text{Brine}}$: $x_1 = (\text{Cl}^-_{\text{Brine}} - 160.8) / (22.5 - 160.8)$. The saturation Cl^- -concentration of Atlantis II brine is 160.8 g per kg at 25 °C (ref. 2); 22.5 g per kg (ref. 6) was determined for recent Red Sea deep water

We conclude that the brines from below the Erba, Port Sudan and Nereus Deep are Red Sea waters, which infiltrated to sub-bottom strata during times when the isotopic composition of the Red Sea was significantly enriched in heavy isotopes as compared with today; that is, these brines are essentially derived from Red Sea palaeowaters.

The same concept could also explain the origin of the Atlantis II Deep brines. During past pluvial periods the Red Sea water was depleted in ^{18}O by up to 1‰ compared to open ocean water¹¹. An ^{18}O -depletion is also indicated in Fig. 3 by the negative $\Delta^{18}\text{O}$ values at inferred ages that are about 5,000 yr BP. We therefore propose that the isotopic composition of the Atlantis II Deep should not be related to present-day Red Sea surface waters but more likely to isotopically depleted Red Sea deep waters from previous pluvial periods. The depletion in ^{18}O of $\sim -0.5\%$ during the last climatic optimum ($\sim 5,000$ – $8,000$ yr BP) as indicated in Fig. 3 is sufficient to explain the isotopic composition of the Atlantis II Deep brines which are also depleted by 0.6‰ as compared to present day Red Sea deep waters.

The concept of a palaeowater origin of the brines in the Red Sea agrees with the widespread occurrence of brines and eliminates the need to hypothesise their derivation from waters to the far south. We assume that these palaeowaters were trapped in an earlier geological period and have been stored since then in subterranean strata. Alternatively, dynamic systems have to be assumed where the subsurface flow times of these systems are of the order of 7,000–15,000 yr.

The brines in the Kebrit and Suakin Deepes do not have isotopic compositions within the range of possible Red Sea palaeowater compositions. Some specific waters of the meteoric water cycle have similar compositions. Such waters were found in lakes in the Danakil Desert¹² or in hot springs in the area around Djibouti¹³. The intrusion of such waters at the bottom of the Red Sea is unrealistic. Therefore, we assume the Kebrit and Suakin brines to be Red Sea palaeowaters which have changed their isotopic compositions due to secondary effects. High temperature exchange reactions between minerals and waters

Fig. 2 Isotopic composition of Red Sea brines and some inferred source fluids. Each brine which has a salinity higher than the Atlantis II Deep brine has a corrected value (○) which is connected with the measured value (●). The corrected values give the original isotopic composition of the brines, if it is assumed that the lower salinities were caused by dilution with present-day seawater. The isotopic variation of world ocean water and Red Sea deep water is shown for the following stages: I interglacial (last climatic optimum 8,000–5,000 yr BP), II present day and III glacial maximum 18,000 yr BP. The isotopic composition of interstitial waters is not well defined (J. R. Lawrence, personal communication); the indicated field encloses the reported analytical uncertainties¹⁶.

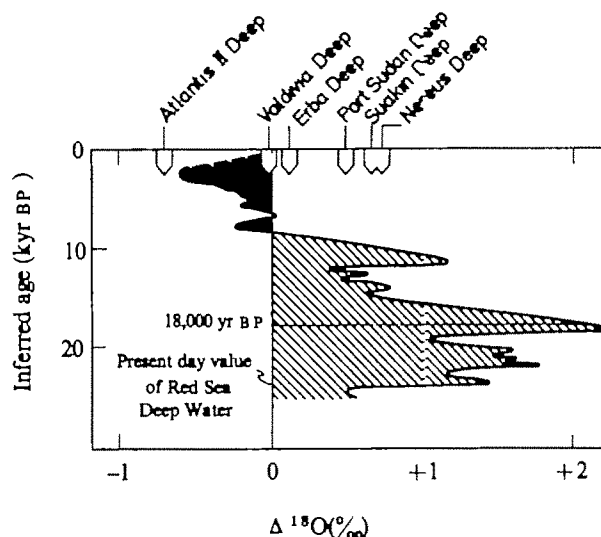
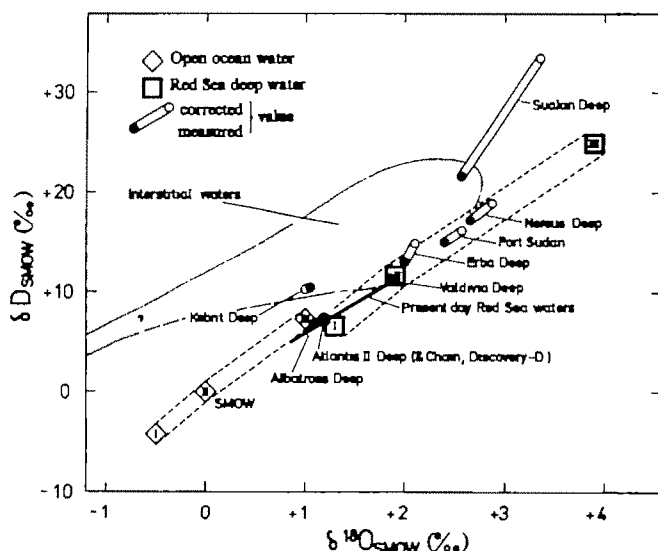


Fig. 3 Variation of the ^{18}O concentration in the Red Sea during the past 20,000 yr. The $\Delta^{18}\text{O}$ values are defined as $\delta^{18}\text{O}_{\text{present}} - \delta^{18}\text{O}_{\text{reconstruct}}$ and show the relative change of ^{18}O in foraminifera in the Red Sea as compared to present day foraminifera values. As the temperature variations in the Red Sea waters were small, the $\Delta^{18}\text{O}$ values represent approximately the variations in ^{18}O in sea water. The time scale has been inferred by assuming the time of maximum ^{18}O enrichment to be 18,000 Yr BP. The ^{18}O concentrations of the brines are given for comparison and are also plotted as differences from present day Red Sea deep water. This comparison shows that the variations in ^{18}O in seawater cover those in the brines.

are improbable. Even the Atlantis II Deep brines, which exhibit the highest temperatures, do not show any ^{18}O isotope shift². We suggest, however, that low temperature mineral reactions, similar to those which alter pore fluid compositions^{14,15} should be considered. Interstitial waters in the Red Sea sediments have been analysed in bore holes near the brine deepes (GC 225 and GC 228 in Fig. 1). Although the ^{18}O concentrations in these fluids could be determined only with great uncertainty (J. R. Lawrence, personal communication), it is important to our results that interstitial waters are similar in their isotopic compositions to those brines which do not fit to proposed unaltered palaeowaters. These waters also tend to be different as compared to Red Sea deep waters and Red Sea palaeowaters and are similar to the brines at the Suakin and Kebrit Deepes. The brines of the Kebrit and Suakin Deep may, therefore, be interpreted as accumulations of expelled interstitial waters.

This interpretation is somewhat speculative, especially taking into account the isotopic composition of the Suakin brines before dilution. However, alternative source fluids like downwards migrated meteoric waters from the Saudi Arabian coast³ are even more speculative and formation waters¹⁸ are ruled out by their isotopic composition.

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Texture discrimination and Fourier analysis in human vision

NEUROPHYSIOLOGICAL experiments have shown that many cells in the visual cortex of the cat and monkey respond well only if the retinal image contains a line stimulus of appropriate orientation and width (spatial frequency)^{1,2}. Different cells are sensitive to different orientation/spatial frequency combinations and judging from numerous corroborative psychophysical experiments, it seems highly probable that similar populations of cells exist in man also³. However, the function of these cells is not known. One suggestion is that they decompose the retinal image into its two-dimensional Fourier components and that processes such as object recognition, region finding and image segmentation then operate on this Fourier description^{4–7}. We report here experimental results on human texture discrimination which argue against this possibility by showing that textures which differ markedly in their Fourier spectrum are not always readily discriminable.

Figure 1 illustrates the texture discrimination task we used. Each stimulus had one 'target' quadrant whose texture differed from that used for the other three quadrants. The stimuli were presented in a tachistoscope and latencies were measured for discrimination of the target. A buzzer warned the subject when a stimulus was about to appear and he indicated his choice for target quadrant by pressing one of four response keys, arranged in a panel to match the layout of the stimulus quadrants. His response terminated the stimulus presentation and also stopped a timer which recorded response latency. Each stimulus was presented four times during an experimental session lasting about 30 min. The location of the target quadrant was varied randomly from trial to trial except that each quadrant was used once for the target for each stimulus. Stimuli were presented in a different quasi-random order for each subject which ensured that he did not know which quadrant was to be the target on any given trial.

Textures consisted of either one sinusoidal grating (45°, Fig. 1a and d), two sinusoidal gratings added together (45° + 105°: Fig. 1b and e), or three sinusoidal gratings added together (45° + 105° + 165°: Fig. 1c and f). In half the stimuli the target quadrant differed from the other quadrants in the spatial frequency of its texture (2.2 and 4.4 cycles per degree respectively: Fig. 1a–c). In the other half the target differed in the orientation of its components (30° rotation: Fig. 1d–f). All stimuli were generated by computer and produced as hardcopy on a full-tone facsimile picture recorder (Muirhead type K-550-B/1).

The reader is invited to judge for himself the difficulty of finding the target quadrant in each of the stimuli shown in Fig. 1. Subjects made the various spatial frequency discriminations very quickly but took much longer over the orientation ones. Even the quickest orientation case (Fig. 1d) produced a mean latency twice as long as any of the spatial frequency ones and in the three-component orientation condition (Fig. 1f) the latency is extremely long indeed.

We have also studied this kind of texture discrimination task using 200-ms tachistoscopic stimulus presentations which prevent benefit from scanning eye movements. Texture discriminations which can be made in such circumstances are

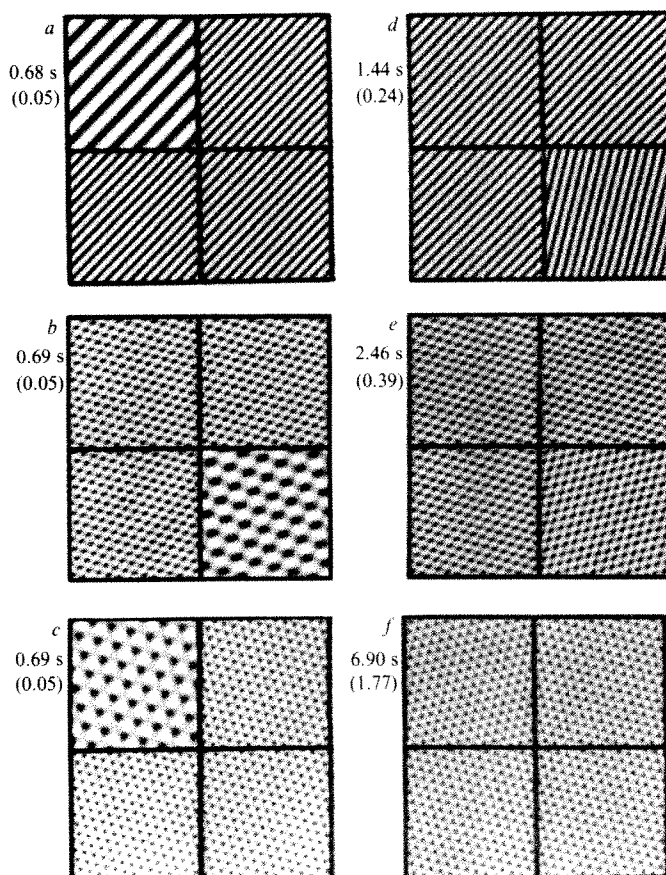
sometimes called 'immediate' to distinguish them from discriminations requiring much more lengthy and careful scrutiny⁸. The spatial frequency discriminations remained easy using 200 ms presentations whereas the orientated type could not be performed reliably, even in the one-component condition⁹. We have also found that these conclusions apply to textures composed of filtered random noise (which appear like woven fabrics).

These results are difficult to reconcile with any theory in which it is assumed that the processes of texture discrimination have immediate access to information corresponding to a local piecewise Fourier analysis. In Fourier terms, the difference between target and non-target textures in all the stimuli of Fig. 1 is clearcut, consisting as they do of widely separated pure sinusoids. Given a Fourier model, therefore, easy discrimination would be expected in all cases but this was not the obtained result.

The results are also difficult to reconcile with Julesz's theory of texture vision¹⁰. Julesz has proposed that textures which differ only in their 3rd or higher order statistics cannot be discriminated without scrutiny. This conjecture does not entail, of course, that all textures differing in their lower order statistics be discriminable. Even so, the second order differences between target and non-target quadrants in Fig. 1 are marked and this makes the relative difficulty with orientated discriminations surprising, given Julesz's basic thesis.

An alternative theory of texture vision has been formulated by Marr¹¹ in which the processes of texture vision have access to a rich but primitive symbolic description of the visual scene called the 'primal sketch'. According to Marr's theory, the primal sketch is computed from local measurements taken at every position in the visual image by the orientated spatial frequency channels. The outputs of these channels are then used to produce a description of the kind of edge or blob present at

Fig. 1 Sample stimuli used in the experiment. Times alongside each figure are group mean latencies ($n = 9$) for detection of the target quadrant (s.e.m. in brackets).



each location, with channel outputs not available to subsequent processes. Consider, for example, Fig. 1c. In terms of Marr's theory, the measurements taken by the various orientated channels are used to form 'assertions', entered into the primal sketch, about the presence of blobs of a particular size and contrast. The clear cut differences in orientated spatial frequency channel activity which would be generated are of no relevance to subsequent processes. Instead, in order to detect the odd quadrant higher-order grouping processes are required to aggregate the blobs into rows. It is only after this grouping process (which would be maximally difficult in Fig. 1f—hence the longest latency for this condition) that the orientation differences in the textures can be detected. On the other hand, in the case of a discrimination based on spatial frequency differences, no such processing is required as the size of the blobs is written directly into the primal sketch and is thus available directly to the processes of immediate texture discrimination. Clearly, our results are compatible with Marr's ideas and challenge any theory which suggests that the outputs of orientated spatial frequency channels are used directly for immediate texture vision.

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Artificial menstrual cycles, behaviour and the role of androgens in female rhesus monkeys

THE sexual behaviour of higher primates has special relevance to the human situation. The rhesus monkey, for instance, has a true menstruation, and copulation occurs throughout the cycle. Levels of sexual activity, however, are generally higher in the peri-ovulatory period¹, and a similar mid-cycle maximum has been reported for certain human couples². Androgens increase the sexual behaviour of rhesus females^{3,4}, and they have been used to treat loss of libido in women⁵. These observations, together with the rise in plasma testosterone near mid-cycle, have led to suggestions that androgens may be the main libidinal hormone in the female⁶. We report here our experiments to test this hypothesis. We devised a daily dose schedule of oestradiol, testosterone and progesterone for ovariectomised rhesus monkeys that duplicated the changing plasma levels during normal menstrual cycles. When males were paired with females so treated, sexual activity showed a pattern similar to that during the normal cycle, and the omission of testosterone was without any detectable effect on this pattern.

Oestrogens given in physiological amounts to ovariectomised female rhesus monkeys increase the sexual behaviour of the pair, whereas injections of progestins antagonise the effects of oestrogen⁷. Testosterone given in pharmacological amounts to ovariectomised females both increases female sexual invitational behaviour⁸ and increases their lever-pressing for access to a male partner in an operant situation⁹. This has raised the question of whether the physiological increase in testosterone,

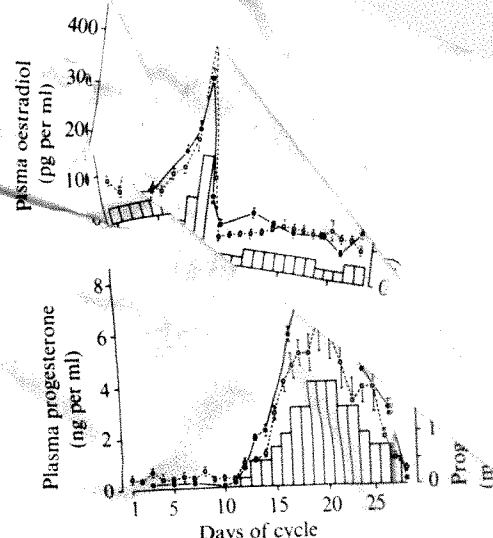


Fig. 1 Doses of oestradiol benzoate (upper histograms) and progesterone (lower histograms) given daily by subcutaneous injection to eight ovariectomised rhesus monkeys to imitate the changing plasma levels during normal cycles. Mean levels of oestradiol (upper graph) and progesterone (lower graph) in plasma samples drawn 8 h after injections during 24 artificial menstrual cycles (●) are compared with mean plasma levels for five intact females during 33 normal menstrual cycles (○). Each point represents the mean of 7–32 values. Vertical bars given standard errors of the means.

rather than the increase in oestrogen levels, near the middle part of the menstrual cycle may be primarily responsible for the menstrual rhythms in sexual behaviour.

Eight female and eight male rhesus monkeys were used in this study. The females were ovariectomised 3 months before the start of the experiment. Doses of oestradiol benzoate, progesterone and testosterone propionate in 0.2 ml sesame oil were administered subcutaneously at 08.00 h each morning according to the 28-d dose schedule shown in Figs 1 and 2. Females so treated had a normal menstruation every 28 d, and we have called these cycles 'artificial menstrual cycles'. Blood samples were collected at 16.00 h three or five times per week, and were analysed for oestradiol, progesterone and testosterone simultaneously using a radioimmunoassay involving chromatography on Sephadex LH20. Changes in the plasma levels of these steroids in 24 artificial menstrual cycles were closely similar to the levels in 33 normal cycles (Figs 1 and 2). Mean values were within the inter-individual variation for this species. When testosterone propionate was omitted from the daily dose schedule (eight cycles), plasma testosterone levels were steady throughout (adrenal secretion) and were similar to the levels in females receiving control injections of vehicle only (Fig. 2b). Thus, the administration of physiological doses of oestradiol and progesterone was without any detectable effect on the secretion of testosterone by the adrenals.

To determine the effect of these dose schedule on sexual behaviour, each female was paired alternately with one of two male partners (groups 1 and 2). Behaviour tests of 1-h duration were observed from behind one-way vision mirrors, and scores were entered directly into a computer through terminals in the observation booths. The number of ejaculations per test is used here as a measure of the males' sexual activity. To measure the females' motivation for propinquity with males, four of the females (eight pairs) were tested in an operant situation that permitted them to press a lever 250 times to gain access to a male partner. The females were allowed 30 min to perform this task and a 1-h behaviour test followed, either immediately, if access was attained, or later the same day, if it was not⁹.

When males were paired with females with artificial menstrual cycles, sexual activity showed a very consistent pattern similar to

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that of the normal cycle. Ejaculatory and operant performance were significantly higher during oestradiol, progesterone and testosterone cycles than during control cycles. Ejaculations increased from 0.90 $\pm$ 0.05 per test ($n = 479$) in control cycles to 1.31 $\pm$ 0.04 per test ($n = 447$) in artificial cycles. The mean access time was 573.1 $\pm$ 55.1 s ($n = 80$) in control cycles and 515.6 $\pm$ 23.6 s ($n = 443$) in artificial cycles. The frequency of ejaculations (0.94 $\pm$ 0.05 per test) was significantly higher in artificial cycles than in control cycles (0.90 $\pm$ 0.05 per test). The mean access time (573.1 $\pm$ 55.1 s) was significantly higher in control cycles than in artificial cycles (515.6 $\pm$ 23.6 s). The frequency of female sexual invitations (presentations, hand-reaches, head-ducks and head-bobs) was significantly higher in artificial cycles than in control cycles. The mean male success ratio (percentage of male mounting attempts not refused by the female) was significantly higher in artificial cycles than in control cycles. The mean male success ratio was 90.5 $\pm$ 2.7% ($n = 65$) in control cycles and 94.5 $\pm$ 2.7% ($n = 443$) in artificial cycles.

Figure 3 shows changes in ejaculations, access times, female sexual invitations (presentations, hand-reaches, head-ducks and head-bobs) and in male success ratios (percentage of male mounting attempts not refused by the female) during these artificial cycles. Cyclic patterns of ejaculation occurred in all 24 cycles when all three steroids were administered and in all eight cycles when testosterone was omitted; there were no significant differences (analysis of variance) between cycles with and without testosterone. Access times were shortest in the week of highest oestradiol dosage in both types of artificial cycle. There were no consistent changes either in female proceptivity as measured by sexual invitations or in receptivity as measured by male success ratios, and there was clearly no evidence for reduced numbers of invitations or decreased male success ratios in cycles without testosterone.

These results are consistent with those of others¹⁰ in suggesting that the mid-cycle increase in plasma testosterone during normal menstrual cycles is of ovarian rather than of adrenal origin. Although functional adrenal glands (a major source of androgen in females) are necessary for the expression of many patterns of behaviour, including those of sexual behaviour¹¹, it seems unlikely that the physiological mid-cycle surge in testosterone plays any significant part in the increase in female sexual motivation at this time. The changes in behaviour observed in

Fig. 2 a, Daily doses of testosterone propionate given by subcutaneous injection (histograms) used in artificial menstrual cycles, and mean plasma levels of testosterone in 24 artificial cycles (●) compared with 33 normal cycles (○). b, Mean plasma levels of testosterone in eight artificial cycles (eight females) during which the schedule of oestradiol and progesterone was continued but in which testosterone propionate was omitted. The horizontal interrupted line represents the mean testosterone level (127.6 $\pm$ 25.7 pg per ml, $n = 45$) during control cycles when vehicle only was injected. Vertical bars give the s.e.m.

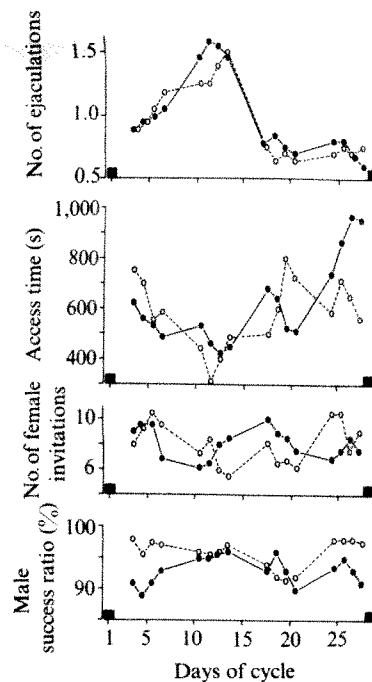
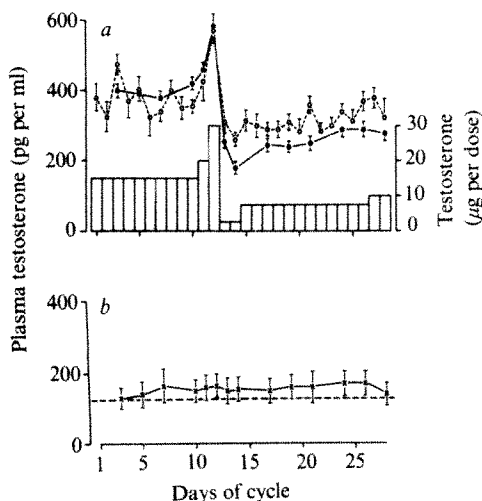


Fig. 3 Changes (means per test) in ejaculations, access times, female invitations and male success ratios during artificial menstrual cycles with oestradiol, progesterone and testosterone (●) and with oestradiol and progesterone alone (○) (2-d running means). Each point represents the mean of 8-48 tests. Black squares indicate menstruation.

the earlier studies cited were produced by doses (0.5-1.0 mg testosterone propionate) that were 15-400 times those used here (2.5-30.0 μ g). These pharmacological doses produce plasma levels in the female rhesus monkey that are well within the normal range for males (4.0-25.0 ng per ml) rather than within the physiological range for females (100-800 pg per ml) that were obtained here. On the other hand, these studies show that normal behaviour cycles result from artificial cycles of physiological doses of oestradiol and progesterone. These findings support the view that the mid-cycle increase in oestradiol is sufficient to account for the increase in sexual activity during the peri-ovulatory period of the normal menstrual cycle.

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Elaborate CNS cooling structures in large dinosaurs

DURING sustained activity, or when exposed to high ambient temperatures, terrestrial animals often experience periods of core hyperthermia. The central nervous system (CNS) is very sensitive to elevated temperatures¹⁻³, and consequently, both bradymetabolic and tachymetabolic⁴ terrestrial vertebrates have evolved physiological mechanisms which effect localised cooling of the brain, and thereby reduce any thermal impairment of CNS functioning. In modern reptiles this temperature gradient is produced by evaporative cooling from the buccal cavity and upper respiratory tract^{5,6}, conducting heat from the brain through the floor of the cranium⁷. Mammals dissipate heat, by evaporation, from the nasal mucosa to the air flowing through the nasal passages. The cooled venous blood draining from this highly vascularised mucosa flows into the cavernous sinus, where counter-current heat exchange with the carotid arteries, elaborated into a rete in many forms, results in brain cooling^{8,9} (Fig. 1a). Dinosaurs would have experienced similar thermal problems to those of modern vertebrates, and these would have been particularly acute in the larger forms whose low surface area to volume ratio would have restricted dissipation of the enormous amounts of heat generated by the skeletal muscles during activity. It is therefore proposed that they required and possessed comparable physiological mechanisms to protect the brain during core hyperthermia.

In the lambeosaurine hadrosaurs, air entering the nostrils passed through extensive passages in a crest formed by the premaxillae and nasal bones before descending the choanal tube to the throat (Fig. 2a). Early workers associated the function of these crests with a semi-aquatic mode of life. The theories proposed included air storage chambers or traps to prevent water entering the lungs¹⁰, but evidence now indicates that hadrosaurs were predominantly terrestrial^{11,12}. Ostrom^{13,14} showed the early theories to be untenable and concluded that the function of the crests was to increase the area of mucosal

Fig. 1 a, Schematic diagram of the blood vessels involved in brain cooling in the sheep (after Hayward and Baker⁹). b, Proposed mechanism of brain cooling in *Edmontosaurus*. Cooled venous blood from the nasal capsule drains into the enlarged orbital sinuses where it conducts heat from the brain.

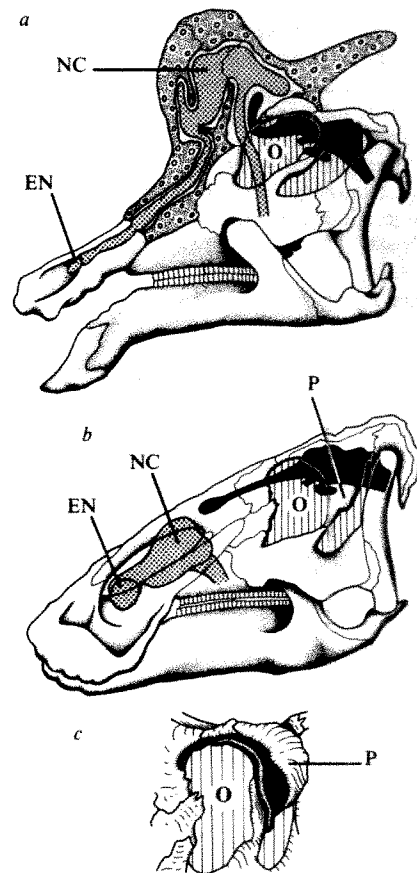
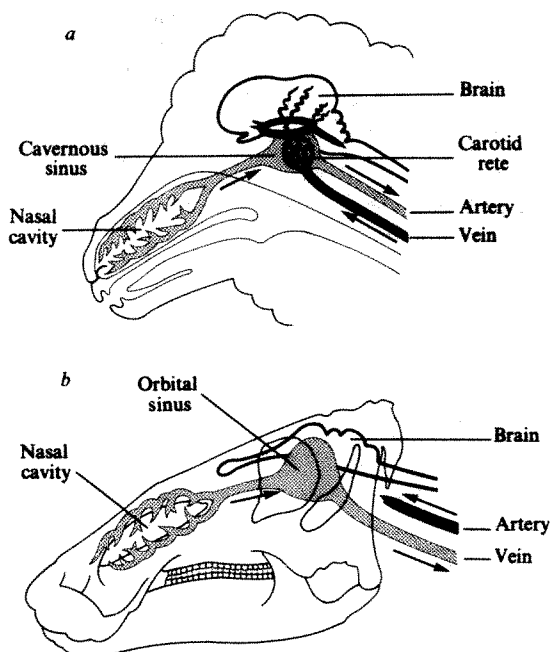


Fig. 2 a, Section through the narial crest of the hadrosaur *Lambeosaurus* illustrating the expanded nasal passages and their position relative to the brain (black). EN, external naris; NC, nasal capsule; O, orbit (after Ostrom¹⁴). b, Skull of the hadrosaurine *Edmontosaurus* showing probable position of the cartilaginous nasal capsule (after Ostrom¹⁴). c, Enlargement of the orbit and postorbital pouches (P) of *Edmontosaurus* (after Ostrom¹³).

epithelium lining the nasal passages, suggesting that this enlargement was for enhanced olfaction. However, it seems likely that the function was primarily thermoregulatory. Although probably too small to have an appreciable effect on core temperature, the enlarged mucosal area would have increased the rate of evaporative heat loss from the head and hence prevented excessive elevation of brain temperature. This idea is supported by the proximity of the passages to the cranium, which would have been cooled by direct conduction into the crest and by the action of the cooled venous blood draining from the mucosa. As in modern mammals, the rate of cooling may have been related to the regulation of air flow through the nasal passages, with panting occurring during heat stress¹⁵. This proposal is not incompatible with the suggestion^{16,17} that these crests, and those of the saurolophine hadrosaurs, also functioned in visual display.

The non-lambeosaurine hadrosaurs lacked hollow narial crests, but their external nares were conspicuously expanded and probably contained an enlarged cartilaginous nasal capsule¹⁴ (Fig. 2b), the lining of the channels within which would have formed an extensive evaporative surface. Unlike the lambeosaurine crests, the location of this surface indicates little influence on brain temperature by direct heat conduction; however, the venous blood draining from it would have cooled the cranium. Evidence for this is the presence, in some genera, of large pouch-like cavities in the postorbital bones (Fig. 2c). Ostrom¹³ suggested these resulted from enlargement of the orbital sinuses, venous structures which in modern reptiles collect blood from the surrounding areas of the head and empty into the internal jugular veins. If this interpretation is correct the cooled venous blood from the nasal capsule would have drained

into these enlarged sinuses, the proximity of which to the cranium would have resulted in brain cooling (Fig. 1b). Furthermore, counter-current heat exchange may have occurred between the jugular venous and carotid arterial blood, similar to that described in modern mammals⁸ and reptiles¹⁸.

Many sauropods greatly exceeded 10 tonnes in weight and were the largest known dinosaurs. The external nares of these animals were situated high on the skull and were usually very large, indicating a spacious nasal capsule (Fig. 3). This location of the nares was thought to have been an aquatic adaptation, allowing these animals to breathe while almost totally submerged¹⁰; but the nares of most aquatic reptiles are small, unlike those of sauropods. From the structure of the vertebral column and limbs, Bakker^{19,20} proposed that sauropods were terrestrial although he was unable to clarify the functional significance of the dorsal position of the nares. However, both the size and location of the nasal capsule are consistent with its having an important role in brain thermoregulation, its enlargement increasing the mucosal area available for evaporation and its position reducing the distance between this cooling surface and the cranium. The long neck of these dinosaurs increased the thermal isolation of the head from the body and extended the distance available for heat exchange between the jugular veins and carotid arteries. This explanation of narial structure strongly supports the evidence that sauropods were terrestrial¹⁹⁻²¹ and argues against the aquatic theory.

As in sauropods, hadrosaurs and large ornithomimid dinosaurs such as *Iguanodon*, the nasal region of the skull of the ceratopsians was greatly enlarged and again the nasal mucosa was probably the main site of heat dissipation. In modern ruminants²² the surface of the bony horn cores is covered with a rich vascular plexus which vasodilates during heat stress, resulting in increased blood flow through the horns. This blood, cooled by convection and radiation from the surface of the horns, drains into the cavernous sinus where it comes into contact with the carotid rete and cools the arterial blood entering the brain. Most ceratopsians possessed one or more horns with cores very like those of ruminants¹⁰. A network of channels on their surface indicates that they too were highly vascularised and therefore could have functioned comparably in brain thermoregulation. Vascularisation of the surface of the ceratopsian

parietal-squamosal neck frills, especially the marginal areas not overlain by jaw musculature²³, would also have permitted additional heat loss from the head.

In the carnosaurs, whose external nares are not as enlarged as those of other large dinosaurs, a major site of heat loss was probably, as in modern reptiles, the lining of their enormous buccal cavity. In some forms there was considerable flexibility between the various jaw elements, structural modifications thought to have facilitated the engulfing of large portions of prey¹⁰. This could indicate the presence of a fairly extensive gular area which would have also further extended the evaporative surface area. Additional cooling may have been achieved by active pumping or fluttering of this gular region, a method used by large varanid lizards²⁴ and birds such as the ostrich²⁵.

Some large dinosaurs evolved structures which can be interpreted as providing thermal protection for the extracranial CNS. The vertebrae of many forms contain deep excavations which probably contained an air sac system similar to that of birds²⁶. Ventilation of this system would have removed heat from the region around the spinal cord. Not surprisingly, the excavations are particularly extensive in the vertebrae of sauropods²¹, which, because of their size, would have been especially vulnerable to hyperthermia. *Stegosaurus* possessed additional radiative and convective structures consisting of a double row of bony plates, well permeated by blood vessels, running along the neck, back and tail²⁷. Although the size of these plates suggests they significantly influenced core temperature, the spinal cord would have been particularly well protected from elevated temperatures by the cooled blood draining from the plates directly above it. The largest plates are located in the sacral region where the spinal cord was apparently enlarged into a ganglion exceeding the brain in size. The vertebral neural spines of a few dinosaurs, such as *Spinosaurus* and *Ouranosaurus*, were extremely elongated and are thought to have supported a vascular sail-like structure which would similarly have permitted increased radiative and convection heat loss, so cooling the spinal region.

The described CNS cooling mechanisms do not constitute direct evidence that dinosaurs were either bradymetabolic or tachymetabolic animals, as such physiological mechanisms are found in the extant groups of both. However, the apparent very highly developed nature of these structures in dinosaurs does tend to support the idea that they may have been tachymetabolic, with a high metabolic level and resulting endogenous heat production, rather than bradymetabolic like modern reptiles. Unfortunately, the nervous and vascular systems are not preserved in fossils and it is only possible to observe the structure of those portions which leave an impression on the skeleton. This makes it impossible to establish the detailed physiological functioning of the proposed cooling mechanisms, but it seems reasonable to assume that they would have been as sophisticated as those of modern vertebrates, incorporating heat-exchanging retes and vascular shunts^{18,28} allowing control of blood flow to and from the cooling surfaces.

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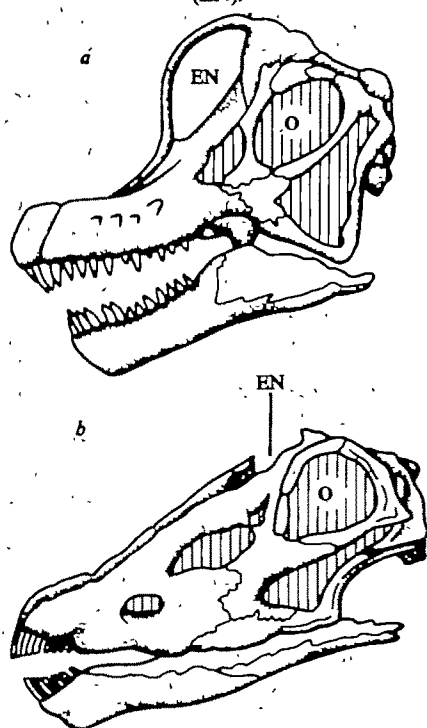
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Fig. 3 Skulls of the sauropods *Brachiosaurus* (a) and *Diplodocus* (b), illustrating the size and dorsal location of the external nares (EN).



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Unusual response of DDT-resistant houseflies to carbinol analogues of DDT

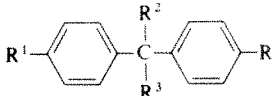
THE toxic action of DDT is thought to be caused by complex formation between the insecticide and nerve protein, a mechanism consistent with the well known phenomenon of negative correlation between insect mortality and temperature¹. We present here evidence which indicates that some carbinols which are close analogues of DDT may have a different mode of action. These compounds elicit very different symptoms of poisoning, and none of the major mechanisms of resistance to DDT in the housefly confers significant resistance to these compounds.

Several analogues of DDT (Table 1) were tested against strains of houseflies (*Musca domestica* L.) with known genetically isolated mechanisms of resistance to DDT. These strains were: Beiruth (heterozygous for *DDTase*), 2778 (*DDT md*, monooxygenase, sesamex-suppressible resistance), 538 ge (*kdr*-resistance)² and Super-*kdr* (*kdr*-like resistance). This last mechanism, not yet fully characterised, was recently isolated from the multi-resistant 153y3 strain of houseflies by the chromosome isolation technique³. Super-*kdr* resembles *kdr* in many respects; it is controlled by one or more incompletely recessive factor(s) on chromosome 3, confers resistance not only to DDT and its analogues but also to the pyrethroids, and is unaffected by pretreatment with FDMC (bis(*p*-chlorophenyl)trifluoromethyl carbinol; 1 µg per fly), piperonyl butoxide (2 µg per fly) or the two additives applied together. Also, it crosses freely with *bwb* (brown body, a recessive mutant marker for chromosome 3), confers no detectable resistance to organophosphorus or carbamate insecticides and differs from *kdr* only in its greater or much greater resistance towards DDT-like compounds and the pyrethroids (Table 2).

Three- to five-day-old female houseflies of the four strains were treated at room temperature with the compounds dissolved in acetone, and were held either for 48 h at 20 °C or 3-4 d at 15, 20 and 28 °C, and were checked daily for numbers killed. Of the carbinols tested (Table 1) only dicofol and FDMC had relatively strong insecticidal activity.

DDT and four of its analogues (Table 1) produced typical symptoms of DDT poisoning in the susceptible and three of the resistant flies. These symptoms are increased activity, tremors,

Table 1 Structure of the DDT analogues tested

Compound			
	R ₁	R ₂	R ₃
<i>p-p'</i> -DDT	Cl	H	CCl ₃
TDE	Cl	H	CHCl ₂
Perthane	C ₂ H ₅	H	CCl ₂
Methoxychlor	CH ₃ O	H	CCl ₃
Ethoxychlor	C ₂ H ₅ O	H	CCl ₃
Chlorphenethol	Cl	OH	CH ₃
Dicofol	Cl	OH	CCl ₃
FDMC	Cl	OH	CF ₃
Chlorobenzylate	Cl	OH	COOC ₂ H ₅
Chloropropylate	Cl	OH	COOCH(CH ₃) ₂

incoordination and tetany. Toxic symptoms were delayed in the resistant flies, particularly in the Super-*kdr* strain, in which they became visible at least several hours after treatment with DDT. The carbinols produced different symptoms which were the same for all the strains tested. The initial sign of poisoning, increased locomotor activity, was first apparent several hours after treatment even at the strongest dose and the highest holding temperature. The insects then exhibited transient collapse with a rapid return to normal activity. The periods of prostration, initially infrequent and very brief, increased in duration and frequency until the insects were no longer able to right themselves or walk. Tremors, incoordination of the limbs and increased wing-beat observed in DDT-poisoned flies were absent or rare in the carbinol-treated insects. Paralysis was neither induced nor reversed by altering the holding temperature, but the insects were killed faster at the higher temperature. At death (end-point), the relative toxicities of the carbinols varied relatively little for the three holding temperatures (Table 3), but those for DDT and its analogues were negatively correlated with temperature (Table 3) in all strains except Super-*kdr*, which had a positive temperature coefficient for DDT (Table 3). This is almost certainly due to the very slow action of this compound against those flies at 20 and 15 °C, temperatures at which death is delayed well beyond 4 d. Perthane, which acts much faster against Super-*kdr* flies than DDT, gave a negative temperature coefficient.

The other major difference with the carbinols was the slight or negligible resistance of the DDT-resistant strains to these compounds (Table 3). This lack of effect by major known mechanisms of resistance to DDT in the housefly was unexpected, particularly so for *kdr*, which is believed to alter either access of insecticide to the site of action, or the site of action itself, presumably a nerve-membrane receptor for DDT-type insecticides¹. (*kdr* confers resistance to DDT analogues that

Table 2 Cross resistance of strain 538 ge and Super-*kdr* to several insecticides 48 h after treatment at 20 °C

<i>LD</i> ₅₀ µg per fly $\pm$ s.e. (resistance factor)									
Insecticide		Susceptible			538 ge			Super- <i>kdr</i>	
Malathion	0.37	1.10	(1.0)	0.40	1.08	(1.1)	0.36	1.09	(0.97)
Parathion	0.016	1.08	(1.0)	0.016	1.06	(1.0)	0.018	1.09	(1.1)
Bioresmethrin	0.010	1.10	(1.0)	0.045	1.08	(4.5)	0.31	1.10	(31)
Cismethrin*	0.0040	1.14	(1.0)	0.072	1.10	(18)	1.5	1.11	(375)
Decamethrin†	0.00049	1.09	(1.0)	0.0069	1.10	(14)	0.30	1.10	(612)
DDT	0.022	1.07	(1.0)	0.48	1.09	(22)	15.0	1.15	(682)

* 5-Benzyl-3-furylmethyl(1*R*,*cis*)-chrysanthemate.

† S-(α -cyano-3-phenoxybenzyl)(1*R*,*cis*)-[3-(2,2-dibromovinyl)]-2,2-dimethylcyclopropanecarboxylate.

Table 3 Response of several strains of houseflies to some DDT analogues at three holding temperatures

		LD ₅₀ µg per fly $\bar{x}$ s.e.														
Housefly strain resistance mechanism		Susceptible (none)			Betruth DDTase			2778 monooxygenase			538 gc kdr		Super-kdr kdr-like			
Compound	Holding temperature															
DDT	15	0.0075	1.07	(1.0)*		**		0.15	1.09	(1.0)	0.68	1.09	(1.0)	36.0	1.32	(1.0)
	20	0.020	1.09	(2.7)		**		0.35	1.11	(2.3)	0.84	1.09	(1.2)	15.8	1.15	(0.44)
	28	0.041	1.10	(5.5)		**		1.12	1.11	(7.4)	1.23	1.11	(1.8)	9.0	1.20	(0.25)
Perthane	15	0.048	1.08	(1.0)	0.21	1.13	(1.0)	0.21	1.11	(1.0)	0.89	1.08	(1.0)	2.45	1.09	(1.0)
	20	0.11	1.08	(2.3)	0.34	1.12	(1.6)	0.80	1.14	(3.8)	1.64	1.09	(1.8)	5.91	1.13	(2.4)
	28	0.25	1.10	(5.2)	—	—	—	1.69	1.09	(8.0)	2.05	1.08	(2.3)	8.38	1.11	(3.4)
TDE	15	0.16	1.07	(1.0)		**		0.26	1.09	(1.0)	1.25	1.08	(1.0)	***		
	20	—	—	—		**		0.63	1.08	(2.4)	2.64	1.11	(2.1)	***		
	28	0.44	1.08	(2.8)		**		—	—	—	4.12	1.13	(3.3)	***		
Ethoxychlor	15	0.029	1.10	(1.0)		**		0.20	1.08	(1.0)	1.59	1.12	(1.0)	***		
	20	0.064	1.07	(2.2)		**		1.85	1.10	(9.3)	13.2	1.31	(8.3)	***		
	28	0.095	1.10	(3.3)		**		—	—	—	***			***		
Methoxychlor	15	0.073	1.06	(1.0)		**		0.86	1.10	(1.0)	1.80	1.09	(1.0)	***		
	20	0.076	1.10	(1.0)		**		2.03	1.09	(2.4)	3.95	1.14	(2.2)	***		
	28	0.12	1.11	(1.6)		**		—	—	—	43.8	1.35	(24.3)	***		
FDMC	15	1.85	1.08	(1.0)	2.09	1.06	(1.0)	2.49	1.08	(1.0)	1.17	1.07	(1.0)	2.92	1.08	(1.0)
	20	2.27	1.09	(1.2)	3.23	1.07	(1.5)	2.68	1.09	(1.1)	2.16	1.09	(1.8)	2.81	1.08	(0.96)
	28	1.65	1.17	(0.89)	2.51	1.09	(1.2)	3.85	1.16	(1.5)	2.26	1.09	(1.9)	2.37	1.11	(0.81)
Dicofol	15	1.07	1.25	(1.0)	2.12	1.10	(1.0)	—	—	—	1.93	1.08	(1.0)	3.79	1.09	(1.0)
	20	1.39	1.14	(1.3)	2.86	1.12	(1.3)	2.6	1.11	—	2.02	1.08	(1.0)	2.69	1.09	(0.71)
	28	1.41	1.15	(1.3)	2.11	1.14	(1.0)	—	—	—	2.47	1.13	(1.28)	2.36	1.09	(0.62)

* Numbers in parentheses indicate temperature coefficient, LD_{50} at given temperature/ LD_{50} at 15 °C.

** Strain heterogeneous for DDTase. Hence, LD_{50} s for insects with strong DDTase not measurable.

*** LD_{50} s not obtained with doses exceeding 40 μ g insecticide per fly.

cannot be dehydrochlorinated and is responsible for low nerve sensitivity to DDT directly applied to the thoracic ganglion⁴). Dicofol and FDMC are therefore not only potent inhibitors of DDT dehydrochlorinase but also affect a site of action different from that primarily affected by DDT. This site is unknown, but several enzymes are known to be affected by DDT⁵, particularly the oligomycin-sensitive Mg^{2+} ATPase⁶. Chang and Cutkomp⁷ have demonstrated that inhibition of this enzyme in cockroach muscle by DDT, TDE, methoxychlor and DDE has a negative temperature coefficient, whereas the activity of dicofol and chlorphenethol increases with temperature.

It is interesting to speculate whether the major target of attack by carbinols in acarines is similar to that in houseflies, as acarines are very susceptible to most of the carbinols tested here but are unaffected by DDT and many of its analogues. Also, it remains to be seen whether the slow toxic action described here is confined only to the carbinol analogues of DDT, as the screening procedures used so far for bioassays on analogues of DDT were not designed to detect this type of action.

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Chloroquine enhances Epstein-Barr virus expression

AFRICAN Burkitt's lymphoma (BL) is a malignant tumour of children most common in areas having holoendemic malaria^{1,2}. It occurs with a particularly high frequency in certain areas of Africa³, with sporadic cases in other areas including Europe and North America^{4–7}. Malaria has been shown to have immunosuppressive effects^{8,9}, and chronic malaria, in particular, causes repeated stimulation of the immune response to infections with plasmodia. It has also been proposed that the immunosuppressive action of malarial drugs may make the immune system more susceptible to cancer and may contribute to BL development. Chloroquine is widely used as an anti-malarial agent and also in the therapy of connective tissue diseases^{10,11}. It is thought to act by forming molecular complexes with plasmodial DNA, causing inhibition of plasmodial DNA synthesis^{12–14}. It has been suggested that chloroquine may play a part in the development of BL¹⁵. Another factor thought to be involved in the aetiology of the African form of BL is the Epstein-Barr virus. Virtually all BLs are EBV genome-carrying tumours and patients have high titres of antibody against EBV^{16,17}. The most widely proposed hypothesis is that BL is due to EBV-induced malignant transformation of thymus-independent B lymphocytes^{17,18}. It has been shown *in vitro* that EBV can induce transformation of resting human and simian lymphocytes which become established as permanent lines^{19,20}. Almost all the cells in these lines contain EBNA (the EBV-induced nuclear antigen) and carry multiple copies of EBV DNA^{21,22}. It has also been shown that EBV can induce lymphomas in experimentally infected monkeys²³. However, the failure of EBV infection to have serious adverse effects in the great majority of humans indicates that interaction with some

other factor, such as malaria or antimalarial drugs, must be involved in BL pathogenesis. We report here studies on the effect of chloroquine on the expression of EBV antigens. The data obtained indicate that chloroquine can activate the EBV cycle and the expression of EBV antigens.

EBV-infected cells express one or more of the EBV-induced antigens, which are readily detectable by immunofluorescence with the use of human sera containing anti-EBV antibodies. These antigens include EBNA, early antigen (EA), virus capsid antigen (VCA) and membrane antigen (MA). The cell line used here was Raji²⁴, which is EBNA-positive, EA-negative and VCA-negative. The cells were grown in medium RPMI 1640 supplemented with 10% heat-inactivated fetal bovine serum, penicillin (100 U ml⁻¹) and streptomycin (100 µg ml⁻¹). Raji cells were superinfected with P3HR-1 strain of EBV²⁵ for the induction of EA and VCA. Drug treatment was carried out as follows: 2×10^6 lymphoid cells were sedimented by low-speed centrifugation and the cells were washed three times with phosphate-buffered saline (PBS). The pellet was dispersed and incubated at 37°C in 5 ml of medium containing chloroquine (diphosphate salt, Sigma) at concentrations of 0, 0.1, 1.0, 5.0 µg ml⁻¹ (0, 1.9×10^{-7} , 1.9×10^{-6} , 9.5×10^{-6} M). Six tubes were prepared for each treatment and the viability of the cells (as determined by the trypan blue exclusion test) was estimated after 20–24 h. Only cell preparations which contained less than 5% nonviable cells were used for EBV infections. 200,000 cells from each of these preparations were pelleted by low-speed centrifugation. The supernatant was discarded and 0.1 ml virus suspension was added per pellet, except to the control. The virus-cell mixture was shaken gently, 1 ml of medium containing the appropriate amount of chloroquine was added and the cultures incubated for 48 h at 37°C. Cells were then collected, washed three times in PBS and resuspended in it. Four smears were prepared from each culture, dried in air at room temperature, and fixed in a 1:1 mixture of acetone-methanol at -20°C for 3 min. The smears were kept at -20°C until used for staining by the standard immunofluorescence methods described in Table 1.

The data on the effect of chloroquine on superinfection of Raji cells with P3HR-1 EBV are shown in Table 1. Chloroquine treatment caused a significant increase in EA-positive cells at a concentration as low as 0.1 µg ml⁻¹ (1.9×10^{-7} M). In this regard, it is interesting that plasma concentrations of chloroquine in man during malaria chemotherapy range from 3×10^{-7} to 10^{-6} M (0.16 to 0.53 µg ml⁻¹)²⁶. Above 0.1 µg ml⁻¹ chloroquine, there was no further increase in the number of EA-positive cells. There was no difference in VCA-positive cells in the presence of chloroquine.

In preliminary experiments we found that chloroquine had no effect on EA and VCA in EBV genome-carrying cells not

superinfected by EBV, thus indicating that unlike iododeoxy-uridine²², chloroquine does not induce antigen expression in non-exogenously infected cells.

The results presented here clearly indicate that chloroquine increases EA expression in superinfected cells. This suggests that chloroquine may be acting either directly on the exogenous EBV genome or indirectly by activating cellular mechanisms essential for the expression of the exogenous EBV genome.

It is well known that BL patients have high antibody titres against EBV antigens^{16,17}. In the present study, chloroquine gave rise to an increased expression of EA, an EBV antigen, which *in vivo* would be expected to provoke an increased antibody reaction. It would therefore be useful to compare levels of antibody titres in BL patients who received chloroquine therapy with control BL patients who were never treated with this drug.

Although the exact mechanism by which chloroquine treatment affects viral expression is unknown, one possibility is that chloroquine alters the relationship between DNA and cellular controls and this hypothesis is now being investigated. Chloroquine can also form molecular complexes with DNA and inhibit DNA-dependent nucleic acid polymerase reactions^{12–14}.

In conclusion, the present study suggests that chloroquine may play an important part in the development of African Burkitt's lymphoma by enhancing the expression of EBV, an oncogenic human herpesvirus. This does not exclude a further contribution resulting from the immunosuppressive action of chloroquine.

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Table 1 Effect of chloroquine on % EA-positive cells

	Raji	Raji+EBV	Raji+EBV+chloroquine (µg ml ⁻¹)		
			0.1	1.0	5.0
EA	0	6.5±0.95	19.0±1.61 (P<0.001)	16.8±1.83 (P<0.001)	18.3±1.97 (P<0.001)

The tests for EA and VCA were carried out by indirect immunofluorescence. The smears were incubated at room temperature for 45 min with anti-EA serum LM (with EBNA-positive, EA-positive and VCA-positive antibody composition) diluted 1:20, and with anti-VCA reference serum JM diluted 1:10. These smears were washed three times with PBS and incubated for 45 min with anti-IgG conjugate (Highland) diluted 1:30. The smears were then washed again three times with PBS, counterstained with Evans blue for 10 min, washed three times with PBS and mounted on a slide in a 1:1 mixture of glycerol-PBS. Raji cells were used as control for EA and B95-8 cells¹⁹ as control for VCA. EA- and VCA-positive cells were estimated as a % of approximately 300 cells. Each concentration was tested in six cultures and the results show the mean ± s.e.m.

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Asbestos-mediated membrane uptake of benzo[a]pyrene observed by fluorescence spectroscopy

EPIDEMIOLOGICAL evidence indicates that particulate matter, such as asbestos, can increase the health risk from exposure to chemical carcinogens. For example, asbestos insulation workers show an eightfold increased disposition towards lung cancer; non-smokers had only a slightly increased risk of lung cancer, whereas the smokers had a 92-fold increased risk^{1,2}. These data indicate that 90% of the asbestos related cancers are the result of the co-carcinogenic effects of cigarette smoking and the inhalation of asbestos fibres. We have now examined one possible mechanism of co-carcinogenesis—the ability of particulate matter to increase the rate of cellular uptake of benzo[a]pyrene (BP). We used fluorescence spectroscopy to investigate the ability of two particulates, silica and asbestos, to adsorb BP, and to deliver the carcinogen to model membranes. The surface area of the asbestos sample was 60-fold less than that of our silica sample. Surprisingly the asbestos sample showed a greater ability to adsorb BP in a monomeric state than silica. Most importantly BP which is adsorbed to asbestos is more rapidly transported into membranes than BP which is adsorbed to silica. Thus increased cellular exposure to carcinogens, resulting from the presence of particulates which can adsorb and deliver these carcinogens to cells, may be involved in the process of co-carcinogenesis.

Figure 1 shows fluorescence emission spectra of BP in dilute benzene solution, and of a suspension of BP crystals in aqueous buffer. A structured emission is observed from BP dissolved in benzene, whereas the suspension of BP crystals yields a broad structureless emission at longer wavelengths. This latter emission results from formation of an excited state charge-transfer complex between two or more adjacent BP molecules³ and is called the excimer emission. The fluorescence emission spectra may therefore be used to reveal the proximity of BP molecules to one another. Also shown in Fig. 1 is the fluorescence emission spectrum of BP which is partitioned into lipid bilayers after uptake from asbestos fibres. Only the structured emission is observed which is indicative of widely dispersed BP molecules.

Fig. 1 Fluorescence emission spectra of benzo[a]pyrene. Emission spectra are shown for BP in benzene (—), and as a suspension of crystals in aqueous buffer (---). After addition of DPPC vesicles to asbestos containing adsorbed BP (· · ·) the emission spectra closely resembles that of BP in benzene.

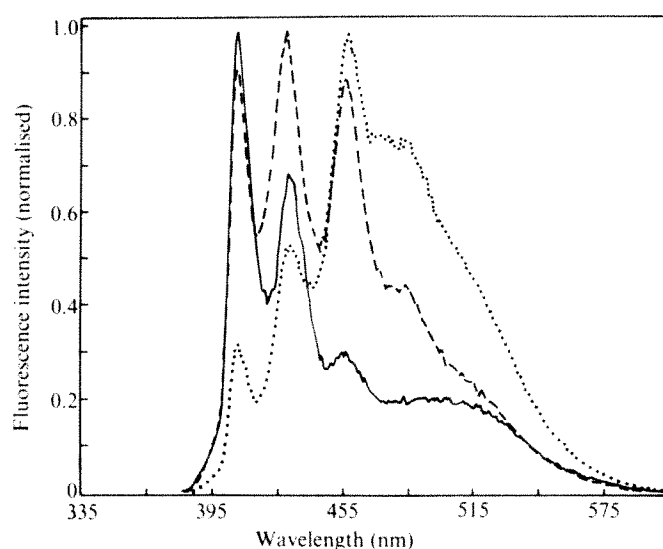
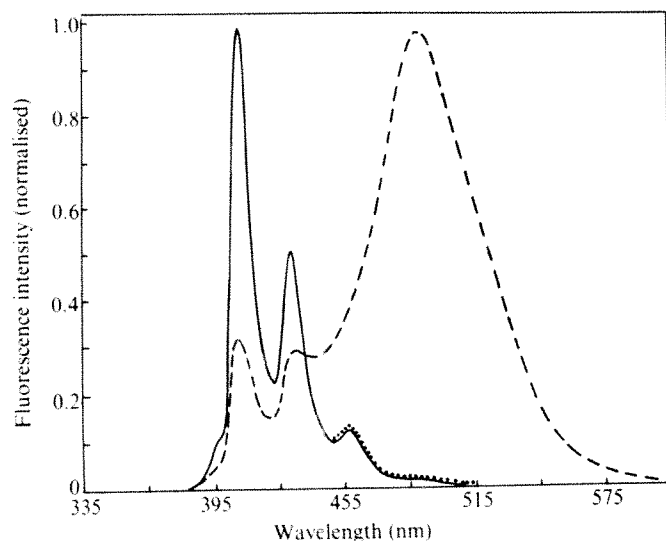


Fig. 2 Fluorescence emission spectra of benzo[a]pyrene. Normalised emission spectra for BP microcrystals (· · ·), and for BP adsorbed to 8.6 mg silica (---) and 9.5 mg asbestos (—). In each case the sample was suspended in 10 ml of buffer, and contained 5 μ g BP.

Figure 2 shows the fluorescence emission spectra of BP when present as microcrystals which are suspended in aqueous buffer, and when the BP is adsorbed to silica or the amosite, which is the form of asbestos used in our studies (see Table 1 legend for experimental details). The emission spectra of the microcrystals and of the silica-adsorbed BP are similar, indicating the presence of BP microcrystals on the silica surface, or the presence of BP crystals which did not adsorb to silica during the solvent evaporation process. The emission spectra of the asbestos-adsorbed BP is dominated by the structured emission, indicating the presence of monomeric adsorbed BP. The superior ability of amosite to disperse BP is especially surprising when the surface areas of these two particulates are compared. As determined by nitrogen adsorption these surface areas are 5.7 and 381 $\text{m}^2 \text{g}^{-1}$ for asbestos and silica, respectively⁴⁻⁶.

The fluorescence emission spectra of BP are sensitive to both the weight percentage of carcinogen which is on the particulate and to the age of the samples. Over a period of weeks after preparation the emission spectra of BP on the silica samples becomes more structured and shows less excimer emission. We do not know what factors control this process, but temperature of sample storage and the presence of residual solvent do not seem to affect the rate of sample ageing. Less significant changes occur with asbestos-adsorbed BP. Irrespective of these considerations we consistently observed more BP excimer emission from the silica than from the asbestos samples. These observations include BP-to-particle weight ratios ranging from 3 to 0.1 mg BP per gram of particulate. For both particulates the excimer emission is less pronounced at the lower weight ratios (Table 1).

Emission spectra of aqueous suspensions of particles which contain BP retain a constant intensity and shape over a period of hours. On addition of phospholipid vesicles, an increase in fluorescence intensity occurs at 405 nm. Simultaneously the excimer emission disappears. After complete transfer of BP to the lipid bilayers the emission spectra of all samples listed in Table 1 become identical to that of BP in lipid bilayers (Fig. 1). Thus, significant decomposition of BP on the surface of the particles did not occur.

BP is only slightly soluble in water, so it seems likely that BP crystals will have a cellular availability which is limited by its rate of solubilisation in the aqueous phase. A particulate which adsorbs and disperses BP in a monomeric state could therefore facilitate BP solubilisation and hence increase cellular availability. The transfer of BP from the particulate's surface to the lipid

bilayer is conveniently quantitated by the increase in fluorescence intensity at 405 nm. The percentage of BP transferred is calculated from

$$\% \text{ BP transferred to membrane} = \frac{I(t) - I_0}{I_\infty - I_0}$$

where I_0 is the fluorescence intensities at 405 nm before addition of membranes, I_∞ is the intensity after complete transfer, and $I(t)$ the intensity at time t .

Figure 3 compares the membrane uptake rate of BP from the three states. As a model membrane we used single bilayer vesicles of dipalmitoyl-L- α -phosphatidylcholine^{7,8}. Asbestos-adsorbed BP shows the most rapid rate of membrane uptake, and the microcrystals of BP the slowest rate. Table 1 lists the time required for 50% transfer of BP for particulate samples with a range of BP-to-particulate weight ratios. Asbestos is more effective than silica at all weight ratios, with the most dramatic effect being observed at the lower ratios. Asbestos or silica, when added to preformed microcrystals of BP in buffer, did not increase the rate of BP membrane uptake or alter the shape of the fluorescence emission spectra of the suspended microcrystals. Therefore, only BP which is adsorbed to the particles displays an increased rate of membrane uptake. These rates increased as surface density of BP on the particulates decreased.

Our results clearly demonstrate that a particulate with a known co-carcinogenic effect is also effective in the dispersal and transport of benzo[a]pyrene. This observation may partially explain the co-carcinogenic effects of particulates and polynuclear aromatic hydrocarbons⁹⁻¹¹. Presumably a carcinogen must enter a cell before transformation, and such entry must involve transport of the polynuclear aromatic hydrocarbon across the plasma membrane. The lungs have mechanisms whereby particulates are cleared, these being phagocytosis by the lung macrophages and upward ciliary action followed by ingestion¹². Particulate matter which increases the rate of carcinogen transport could therefore increase the effective dose of carcinogen before clearance by these protective mechanisms. This speculation is supported by observations which indicate that soot particles which have been isolated from human lungs no longer contain BP¹³.

Particle-enhanced transport of carcinogens is unlikely to be the only mechanism of co-carcinogenesis. Studies with experimental animals have shown that the inhaled particulate haematite is co-carcinogenic with the injected systemic carcinogen diethylnitrosamine¹⁴. The tumours are localised in the lungs, which are the sites of particle deposition. However, particulate

matter is unlikely to facilitate carcinogen uptake in these conditions where exposure to particulates and carcinogens is by different routes of administration.

Epidemiological studies have correlated human exposure to asbestos with the incidence of mesothelioma, but this cancer

Table 1 Benzo[a]pyrene uptake by phospholipid vesicles from particulate adsorbed and microcrystalline states

mg BP per g particulate	Time for 50% uptake (min)		(Monomer emission) [†]	
	Silica	Asbestos	Silica	Asbestos
0.1	4.5	3	0.32	0.34
0.3	24	6.5	0.24	0.34
1.0	30	12	0.19	0.28
3.0	34	17	0.23	0.22

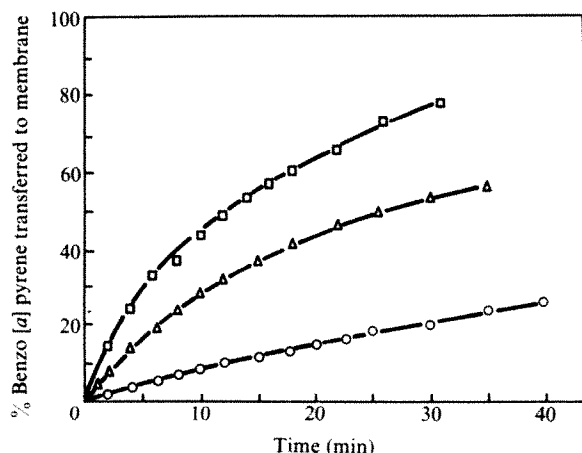
BP Microcrystals	Time for 15% uptake (min)	(Monomer emission)
		(Total emission)
No particles	23	0.17
+ Amosite*	24	0.21
+ Silica*	24	0.19

Benzo[a]pyrene (BP) 99 + % purity was from Aldrich lot 101847 used without further purification. BP concentrations were obtained using a molar extinction coefficient of $57,500 \text{ M}^{-1} \text{ cm}^{-1}$ at 299.4 nm. Silica was obtained from preparative thin-layer chromatographic plates, $20 \times 20 \text{ cm}$, $1,000 \mu\text{m}$ thick (Analabs). The surface area of this sample, as determined by nitrogen adsorption, was $381 \text{ m}^2 \text{ g}^{-1}$. The particle size distribution is heterogeneous, with most particles being between 1.5 and $15 \mu\text{m}$ in diameter, and an average size of about $3 \mu\text{m}$ (ref 6). Amosite served as reference asbestos sample (International Union Against Cancer, Johannesburg). The surface area determined by nitrogen adsorption is $5.6 \text{ m}^2 \text{ g}^{-1}$. The particle size distribution is heterogeneous, ranging from 1.5 to $10 \mu\text{m}$, with the average size $2 \mu\text{m}$. For more detailed information on size and chemical composition see ref. 5. Particulate samples containing BP were prepared by mixing the particulate with a benzene solution of BP, followed by evaporation of the benzene under reduced pressure. 0.1 to 3.0 mg of BP was added for each g particulate. The volume of benzene added was always adequate to wet the entire particulate sample, typically 5 or 6 ml of benzene per g of particulate. The sample was rotated continuously during the benzene evaporation. After the sample appeared dry it was kept under vacuum for 30–45 min at 85°C . At the higher weight ratios BP crystals could be seen on the walls of the glass vessel. Any material which clung to the walls was discarded. After removal from the vessel the particulates were stored in the dark under an argon atmosphere. The amount of BP present on the particles was determined by solvent extraction using a Soxhlet extractor. Typically the extraction was continued for 4.5 h (about 50 individual extractions). All extractions were done in duplicate. The fluorescence emission spectra of the extracted material were identical to that of the starting material. Thus there seemed to be no degradation of BP on this particulate which interfered with our measurements. The amounts of BP adsorbed to silica and asbestos, were found to be $85 \pm 15\%$ of the amount added. Aqueous dispersions of BP crystals were prepared by evaporation of a benzene solution of BP to dryness, addition of buffer, followed by sonication for 30 min at 40 W using a Cole-Parmer Model 8845-2 water bath-type sonicator. We will refer to this preparation as being microcrystalline. In all studies the buffer was 0.01 M Tris-HCl, 0.05 M KCl, $\text{pH} = 7.5$. Phospholipid vesicles of dipalmitoyl-L- α -phosphatidylcholine were prepared by ultrasonic irradiation of the lipid suspended at 10 mg ml^{-1} . Sonication was performed using a Heat Systems Model 350 for 10 min at $\sim 50^\circ \text{C}$. Power input was $\sim 200 \text{ W}$. Vesicles were then centrifuged at $54,000 \text{ g}$ for 60 min. All fluorescence spectral data were obtained on an SLM Instruments, computerised photon counting spectrofluorometer (excitation wavelength, 296 nm; excitation and emission band-passes 8 and 4 nm, respectively; excitation filter, Corning 7-54; emission filters, 0-52 and 2 mm of 1 M NaNO_2). BP uptake was measured by the intensity increase at 405 nm. The emission filters do not transmit light below 395 nm. Without these filters we do observe a background signal from the asbestos and silica. Some background signal was seen above 395 nm, typically $<10\%$ of the total intensity. These backgrounds were quantitated using particles without BP, and subtracted from the spectra shown. Spectral data were obtained using a $2 \times 2 \text{ cm}$ cuvette, 4 cm high, which was thermostated and positioned on a magnetic stirrer so that the stirring bar stayed below the light path. Stirring was required to keep the particles or crystals in suspension. The exciting beam impinged upon the sample at an angle of 20° , and the emission observed from this illuminated surface was at 90° to the exciting light. For all the BP uptake kinetics reported here we used $5 \mu\text{g}$ of BP and 10 mg of DPPC. An amount of particulate containing $5 \mu\text{g}$ BP, or $5 \mu\text{g}$ BP crystals, was suspended in 10 ml of buffer. After measurement of the initial intensity 10 mg of DPPC were added in 1 ml of buffer to initiate the reaction. All measurements were performed at 25°C . Complete BP transfer to the bilayers was obtained by heating of the sample to 55°C for 30 min. The final fluorescence intensity was measured after equilibration at the experimental temperature of 25°C .

* Particles were added after formation of the microcrystals.

[†] This value was estimated from the ratio of area under the fluorescence emission spectrum from 380 to 440 nm to the total area from 380 to 605 nm. After complete uptake of BP by vesicles this ratio is 0.47.

Fig. 3 Rate of benzo[a]pyrene uptake in to lipid bilayers. The % of benzo[a]pyrene transferred to DPPC vesicles is shown for microcrystals of BP (○), silica adsorbed BP (△), and amosite adsorbed BP (□).



does not seem to be correlated with cigarette smoking¹⁵. Particulates such as fibrous glass¹⁶, asbestos^{10,11} and many other types of foreign bodies¹⁷ seem to be carcinogenic without deliberate exposure to chemical carcinogens. In fact, most types of cellular trauma and scarification will predispose the damaged areas to neoplastic transformation^{18,19}. Therefore it is clear that mechanisms other than particle enhanced transport are operative in carcinogenesis. The fluorescence methodology described here is useful with other polynuclear aromatic hydrocarbons⁶ and with nitrogen heterocyclic compounds²⁰. The ability to quantitate the delivery of carcinogens to cells should aid in elucidating the multiple etiology of carcinogenesis.

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Electromechanical properties of articular cartilage during compression and stress relaxation

THE main physiological function of articular cartilage is to act as a bearing material. It consists of an organic matrix made of collagen, proteoglycans, non-collagenous proteins, lipids and cells that are swollen with water^{1,2}. Its net fixed charge is negative and directly proportional in magnitude to its hexosamine content at each depth³. When cartilage is compressed, interstitial fluid is exuded. This flow of fluid with respect to the solid matrix plays an important part in controlling various rheological properties of the tissue, such as its creep deformation under load^{4–11}. Also, we have observed that measurable electrical potential differences are induced between the surface and deepest regions of cartilage when it is compressed. We report here our studies of this phenomenon to quantitatively relate the magnitude, sign and time dependence of the electrical potentials to the known features of cartilage mechanics and fluid flow¹¹, that is, in terms of tissue structure, function and composition. Our results suggest that an electrokinetic mechanism is primarily responsible for the mechanical-to-electrical transduction response.

Mechanically induced potentials in cartilage have been observed previously^{12,13} when a load was applied to one region of a hydrated specimen while electrodes were generally located

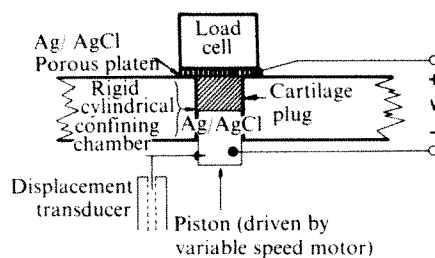


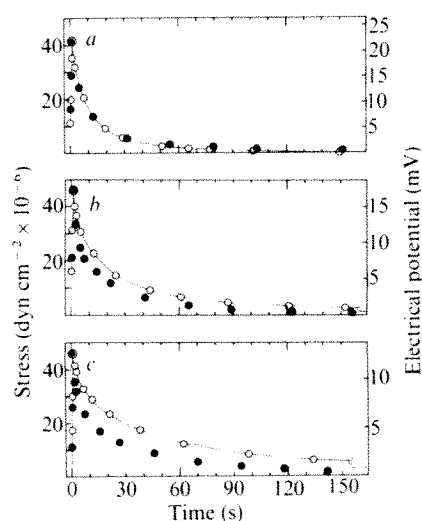
Fig. 1 Experimental configuration for measurement of electrical potential V_0 and mechanical stress σ due to an imposed strain ϵ and strain rate $\dot{\epsilon}$.

in an adjacent region. However, in these experiments the mechanical stress, strain and the fluid velocity field were so complex that it was difficult to relate quantitatively any induced electrical field to the mechanical fields. In our studies, therefore, a geometrical configuration was chosen so as to induce and measure electric fields that are colinear with the axial strain field. This enabled us to compare nonequilibrium electromechanical and mechanical rate (for example, transport) processes.

Circular disks of articular cartilage were compressed in a confining chamber with a chlorided silver rod against a porous Ag/AgCl platen (Fig. 1). The articular surface was positioned against the platen. During and after compression, the stress σ and the potential difference V_0 were measured. (The latter was monitored continuously with a Keithley 602 electrometer and a Tektronix 555 oscilloscope.) When final, time-invariant values of σ and V_0 were attained, the tissue was further compressed to a new strain, at the same strain rate $\dot{\epsilon} = 15\%$ per sec, and the procedure was repeated. Strains in the range of 5–20% were attained with each compression, corresponding to compressions of ~ 0.06 – 0.2 mm. These strains resulted in peak stress levels for normal plugs that ranged over 250–650 p.s.i. (17 – 44×10^6 dyn cm⁻²). The apparatus was a modification of that used previously¹⁴ to study stress relaxation behaviour of articular cartilage.

To ensure that the measurements reflected the properties of the full thickness of the tissue, 40 plugs of cartilage and bone were cut from the parapatellar region of 14 medial femoral condyles of adult steers using a previously described procedure¹⁵. The underlying bone was then removed around the tide mark; the resulting cartilage plugs were 2.6 mm in diameter and ~ 1.0 – 1.5 mm thick when fully hydrated. (However, plugs with a ≥ 1 mm region of bone gave similar results.) The plugs

Fig. 2 Potential (●) and stress (○) history for three successive compressions (a–c) of the same normal plug. ϵ , Cumulative strain: 12.7% (a), 26.4% (b) and 39.4% (c). $\dot{\epsilon}$, Strain rate = 15% s⁻¹ (a–c).



were prepared from tissue that was previously frozen. To assess the effect of such storage, five fresh plugs were prepared from one condyle and tested within 3 h of killing the animal, during which time the tissue was kept at 5 °C. Experiments were also carried out with nine plugs that had been partially digested with testicular hyaluronidase. The conditions used decreased the hexosamine content of the tissue by ~75% (ref. 16), and hence reduced its net negative charge. The plugs were equilibrated in 0.15 M NaCl at 4 °C for at least 1 h before use; longer equilibration times gave the same results. Also, one series of both the normal and enzymatically digested samples were equilibrated in 0.15 M NaCl at pH 2.8, and another series of normal plugs was equilibrated with 5 M NaCl at neutral pH.

The pertinent features of the electromechanical response are typified by the data of Figs 2 and 3. Figure 2 shows the time history of V_0 and σ for three successive compressions of a disk of cartilage equilibrated in 0.15 M NaCl at neutral pH. During the compression phase, $t < T$, $V_0(t)$ was positive and increased linearly with $\sigma(t)$ until time $t = T$ when compression was stopped. The ratio (V_0/σ) at $t = T$ always decreased monotonically with successive compressions at the same strain rate, $\dot{\epsilon}$ (Fig. 2). During the relaxation phase, $t > T$, both $V_0(t)$ and $\sigma(t)$ decreased; the decay with time of V_0 and σ was almost identical after the first compression. With each successive compression, these decay times were longer, but $\sigma(t)$ decayed at a successively slower rate than $V_0(t)$. Although the asymptotic stress was usually finite and progressively larger at the end of each successive compression relaxation cycle, V_0 typically relaxed to zero. These data were obtained with plugs that had been stored in the frozen state, but the results obtained with fresh samples were qualitatively identical.

The compression of enzymatically treated plugs equilibrated at 0.15 M NaCl, neutral pH, resulted in potentials positive in sign but reduced in magnitude by 45–75% (Fig. 3a). A similar result was obtained with normal plugs that had been equilibrated in 0.15 M NaCl, pH 2.8. However, all plugs that were enzymatically treated and equilibrated at pH 2.8 gave negative $V_0(t)$ values (Fig. 3b). Finally, the compression of normal plugs equilibrated in 5 M NaCl resulted in potentials that were drastically reduced in magnitude.

A comparison of the electrical and mechanical rate processes that were highlighted in this study suggests the importance of interstitial fluid flow in the observed electromechanical coupling phenomena. The movement of fluid and concomitant matrix reorientation is thought to control the stress relaxation of articular cartilage^{11,14}. Here, the sign and time dependence of V_0 on pH and neutral salt concentration suggest that the potentials are directly related to the flow of fluid and entrained counter-ions with respect to the net negatively charged solid matrix, that is, that an electrokinetic mechanism is dominant.

In the absence of other mechanisms, an electrokinetic model would predict the relaxation of the potential to zero on cessation of fluid flow, as was seen. Further, the observed decrease in (V_0/σ) at $t = T$ with increasing $\dot{\epsilon}$ is consistent with the known¹⁷ increase in fluid flow resistance at higher $\dot{\epsilon}$. The instances of negative V_0 (Fig. 3b) would indicate that the combination of proteoglycan extraction and carboxyl group neutralisation changed the sign of the net matrix charge from negative to positive. Furthermore, the decrease in V_0 at high NaCl concentration is consistent with a screening of the effective fixed charge by mobile neutral salt ions, that is, a decrease of the electrokinetic ζ potential. Similar effects of pH and ionic strength have been seen with collagen membranes^{18,19}. The effect of pH on the dissociation of proteoglycan and collagen charged groups has also been reported^{20,21}. These results^{18–21}, together with the fact that streaming potentials have been induced *in vitro* using an applied pressure gradient but without varying the strain²², also support our conclusion.

Although electrochemical processes may contribute to the measured V_0 (for example, changes in interfacial Donnan equilibrium potentials, or nonequilibrium diffusion potentials due to strain-induced concentration gradients), their contribution was

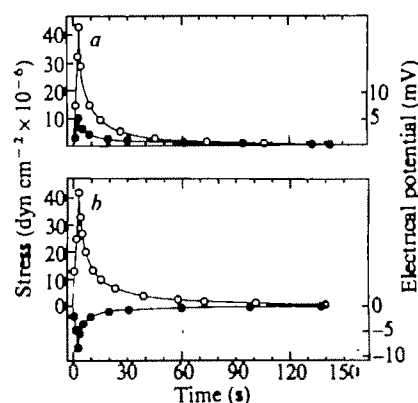


Fig. 3 Potential (●) and stress (○) history with a, enzymatically treated plug pre-equilibrated in 0.1 M NaCl at neutral pH ($\epsilon = 18\%$). b, Enzymatically treated plug pre-equilibrated in 0.1 M NaCl, pH 2.8 ($\epsilon = 34\%$). $\dot{\epsilon} = 15\% \text{ s}^{-1}$.

probably minimal. This emphasises the importance of considering the kinetics of $V_0(t)$ and $\sigma(t)$. For example, an estimate of the decay of a nonequilibrium concentration gradient and its concomitant diffusion potential, characterised by the diffusion time $\tau_{\text{diff}} \sim \delta^2/2D$, is almost two orders of magnitude slower than that observed for the decay of V_0 (based on a typical specimen thickness δ and internal diffusion coefficients D (ref. 23)). On the other hand, the Donnan interfacial potentials span a distance of a few Debye lengths²⁴. They can re-equilibrate in a time which is of the order of the Debye space charge relaxation time²⁵ (the interstitial fluid dielectric constant divided by its conductivity). This is a negligibly short time on the scale of $V_0(t)$, for relevant ranges of parameter values. On the other hand, the hypothesis that relative fluid flow is intimately linked not only to stress relaxation but to the induced potential V_0 is consistent with the observed similarity of the relaxation of $\sigma(t)$ and $V_0(t)$. This, in addition to our other results, lends further support to the notion that an electrokinetic effect is the dominant rate-limiting process.

The occurrence of mechanically induced electric fields *in vivo*, under equivalent physiological loads, may play an important part in regulating the transport of ions and interstitial fluid. We are now trying to assess quantitatively the influence of chemical modifications of the charge groups within the tissue in order to experimentally and theoretically characterise electromechanical influences on transport. Preliminary results show that compression of samples whose carboxyl groups were blocked (using established reactions²⁶) produces a V_0 that is consistently negative. Finally, we note that the successively differing relaxation rates found for stress as compared with potential (Fig. 2) may provide the first evidence and an important method for assessing the relative contributions of the fluid and solid phases to stress relaxation.

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Naloxone reversal of endotoxin hypotension suggests role of endorphins in shock

It is known that the cardiovascular system is extremely sensitive to the effects of both exogenous^{1,2} and endogenous³ opiates. In rats, less than 1% of the morphine dose necessary to produce antinociception results in significant hypotension and bradycardia⁴. The endogenous opiate β -endorphin is stored along with pituitary adrenocorticotrophin (ACTH)^{5,6}, and the action of stressors seems to result in the release of both peptides^{5,6}. Therefore, it seemed likely that β -endorphin is released during shock states and that it might contribute to the hypotension. To test this hypothesis we used an endotoxin shock model^{7,8}. If endotoxin-induced hypotension were mediated through endorphin release, then blockade of endorphins should reverse such hypotension. Using the specific opiate antagonist, naloxone, we not only rapidly reversed endotoxin-induced hypotension, but also prophylactically blocked its occurrence. These findings suggest that endorphins may have a role in the pathophysiology of shock and that narcotic antagonists should be evaluated for their potential therapeutic value in the treatment of shock.

All studies were carried out using conscious rats with chronically indwelling arterial and venous catheters. Two days before experiments, male Wistar rats (Walter Reed strain) weighing 275–300 g were anaesthetised with pentobarbital 50 mg per kg. The tail artery was cannulated by the method of Chiueh and Kopin⁹ for subsequent measurement of blood pressure and heart rate. The external jugular vein was cannulated to allow for intravenous drug injections. Both cannulae were subcutaneously implanted so that they emerged at the back of the neck. The animals had access to food and water ad libitum and were maintained at 24 °C on a 12 h light–dark cycle.

Rats were studied in their home cages with arterial lines connected to a microtransducer (Narco Bio-Systems, model RP1500). Injections of naloxone hydrochloride (Endo Laboratories) or saline were made after administration of *Escherichia coli* lipopolysaccharide endotoxin (Difco, control no. 598159); all injections were given intravenously followed by a 0.3 ml saline intravenous flush to ensure that all of the drug was infused. Blood pressure and heart rate were continuously recorded on a Beckmann dynograph (type R).

Figures 1 and 2 represent the effects of treatment after injection of 4 mg endotoxin per rat. Usually within the first 5–15 min after endotoxin a precipitous fall in blood pressure occurred. When the mean arterial pressure (MAP) dropped to

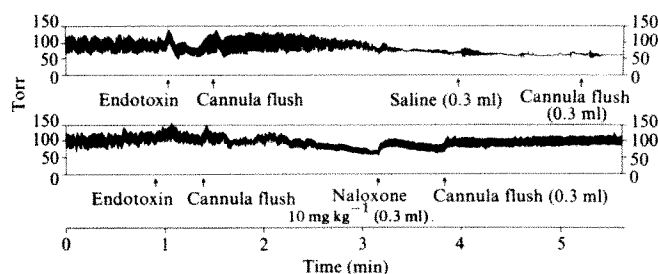


Fig. 1 Effects of intravenous saline (top) and naloxone (bottom) on the precipitous fall in blood pressure produced by 4 mg endotoxin in representative rats. Either saline (0.3 ml) or naloxone was injected when MAP fell to 65–70 mm Hg (torr). The prominent effect of the cannula flush (0.3 ml, bottom) is related to the clearing of residual naloxone from the line.

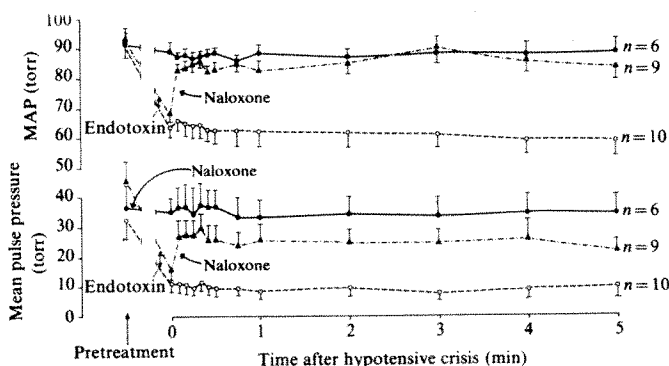
our pre-established criterion of 65–70 mm Hg (torr)⁸, an equivalent volume of either saline or naloxone 10 mg per kg was given by intravenous bolus.

Figure 1 shows the effects of saline and naloxone on endotoxin-induced hypotension in representative rats. In marked contrast to saline, which had only a minor transitory effect (Fig. 1, top), within 5 s after naloxone injection both MAP and pulse pressure were restored to pretreatment values (Fig. 1, bottom). The striking differences between naloxone-treated and saline control rats are further demonstrated by grouped data shown for the 5-min interval after endotoxin-induced hypotension (Fig. 2); again, a rapid return of MAP and pulse pressure towards control levels can be seen following naloxone injection. Naloxone in the absence of endotoxin (Fig. 2, solid line) was without effect on either MAP or pulse pressure.

A comparison of the effects of naloxone pretreatment with saline controls over a 2-h period after endotoxin administration is seen in Fig. 3. Due to the short biological half life of naloxone¹⁰, the initial 10 mg per kg dose of this drug was repeated every 30 min; saline controls were also treated at these same intervals. Although the same general pattern of changes was observed in both groups (Fig. 3), the saline control animals exhibited two hypotensive episodes (10 and 45 min after endotoxin administration) during which blood pressure values were significantly lower than before endotoxin (repeated measurement analysis of variance using Hotelling's T^2 statistic¹¹, $F = 12.29$, $P = 0.003$). By contrast, naloxone-pretreated animals did not show significant declines in blood pressure after endotoxin administration ($F = 0.83$, $P = \text{NS}$).

The reversibility of endotoxin-induced hypotension over a 2-h period is represented in Fig. 4. After injection of 8 mg endotoxin, animals were randomly divided into two equal

Fig. 2 Results of various drug injections on mean arterial blood pressure (top) and mean pulse pressure (bottom). Measurements at $t = 0$ began 5 s after intravenous catheter flush. Naloxone alone (●) did not significantly change either measure. Endotoxin 4 mg intravenously resulted in a significant drop in MAP and pulse pressure from control levels (Student's t test, $P < 0.01$ both groups). Subsequent naloxone injection reversed the drop in MAP and pulse pressure (▲), whereas saline injections (○) were without effect. Points represent averages $\pm$ s.e.m. Naloxone doses 10 mg per kg.



groups. One-half received naloxone 10 mg per kg and the other half saline; these were given only after individual MAPs had dropped to 65–70 torr. Animals receiving naloxone after endotoxin-induced hypotension had significantly higher MAP than those receiving saline (analysis of variance¹², $F = 10.55$, $P = 0.009$).

In addition to blood pressure effects, heart rate was also measured. In the saline control group, heart rate data tended to parallel the blood pressure changes, the lowest average heart rate being observed 55 min after injection (347 ± 33 beats per min, $n = 4$) followed by an increase to significantly higher values at 120 min (453 ± 17 beats per min, $n = 4$, Student's $t = 2.86$, $P < 0.02$). In naloxone-pretreated rats, subsequent injection of endotoxin had no effect on heart rate.

The results of this study clearly demonstrate that naloxone not only blocks but also reverses the hypotensive effects of endotoxin. Heart rate changes after endotoxin also seem to be

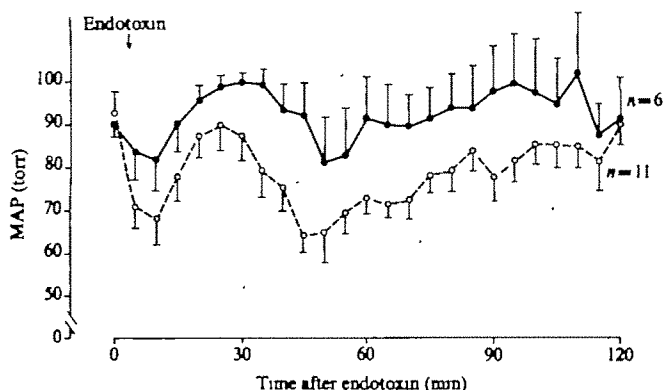


Fig. 3 Effects of naloxone pretreatment on 2 h MAP measurements after endotoxin. Pretreatment with naloxone 10 mg per kg (●) blocked the effects of 4 mg endotoxin on MAP. The groups treated with 4 mg endotoxin alone (○) had significantly lower MAP values compared with pre-endotoxin control values (see text). Vertical bars are $\pm$ s.e.m.

blocked by naloxone. During these experiments the administration of naloxone was discontinued after 2 h (Fig. 3), and survival was not affected. Studies are in progress to evaluate the effects of more prolonged naloxone administration.

The efficacy of the specific opiate antagonist, naloxone, as a modifier of physiological and behavioural events has been used as a tool to infer endorphin involvement in various systems^{13–15}. From that perspective, the effects of naloxone in these present studies implicate endorphin as a factor in endotoxin shock. Interestingly, it has often been suggested that bacterial endotoxins result in the secondary release of endogenous substances which, in turn, may cause some of the cardiovascular effects associated with this form of shock^{7,8}. Our studies suggest that endorphin may be one of these postulated substances.

Endotoxins are known to result in the secretion of ACTH from the pituitary, presumably by activation of hypothalamic releasing factors¹⁶. It has been shown recently that both ACTH and β -endorphin are stored in common pituitary sites and concomitantly regulated^{5,6}. It seems probable that endotoxins would therefore also release pituitary β -endorphin. Moreover, it seems likely that the established therapeutic efficacy of synthetic corticosteroids^{3,17} in the treatment of shock may be a partial consequence of their inhibitory effects on pituitary β -endorphin release⁵. The enhanced sensitivity of adrenalectomised animals to shock states¹⁸ as well as to intravenous β -endorphin injections¹⁹, both of which are attenuated by corticosteroid pretreatment, are also consistent with the hypothesis that endorphin release is important in the pathophysiology of shock.

The effect of naloxone in reversing the early hypotension of endotoxin shock suggests that naloxone may be efficacious in the

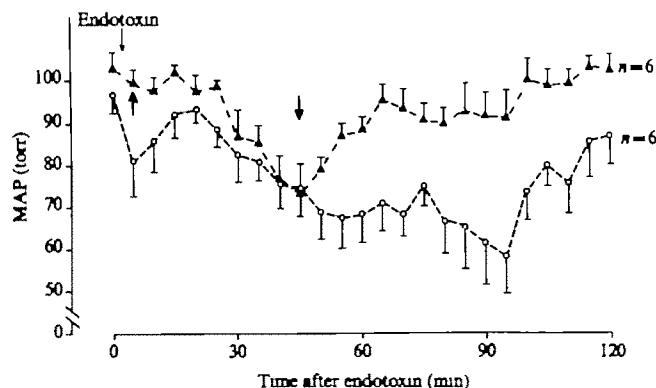


Fig. 4 Reversal of endotoxin shock by naloxone. After injection of 8 mg endotoxin, rats were injected with naloxone 10 mg per kg (▲) or saline (○) after MAP reached 65–70 torr. Large arrows indicate approximate times of naloxone injection. Some animals required two naloxone injections because the hypotensive effects produced by endotoxin were biphasic. Naloxone injections significantly improved MAP (see text). Vertical bars are $\pm$ s.e.m.

treatment of septic shock. The possibility that the endogenous opiates may also be involved in the pathophysiology of other shock models is currently being investigated in our laboratory.

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Nicotinic cholinergic stimulation increases cyclic GMP levels in vertebrate skeletal muscle

CYCLIC GMP seems to be an intracellular second messenger for certain actions of acetylcholine at muscarinic receptors (for a review, see ref. 1). For example, previous studies have provided evidence that activation of muscarinic, but not of nicotinic, cholinergic receptors leads to an increase in the level of cyclic GMP in many tissues^{2–8}, including smooth muscle^{3–5}. Also, it has been demonstrated that contraction of cross-striated muscle fibres of the giant barnacle elicited by nerve stimulation is associated with an increase in the level of muscle cyclic GMP⁹.

These observations prompted us to determine whether a similar cyclic GMP increase is associated with contractile activity in vertebrate skeletal muscle. We report here that activation of a nicotinic cholinergic synapse raises the level of cyclic GMP in frog skeletal muscle. As in smooth^{4,5} and in barnacle⁹ muscle, this cyclic GMP increase in vertebrate skeletal muscle is a consequence of a neurotransmitter-induced depolarisation of the muscle membrane.

The gastrocnemius muscles of 3–4 inch *Rana pipiens* (northern variety) were used in this study. The cyclic nucleotide content of resting muscles from different frogs varied several-fold, but the levels in the two muscles from a single frog were essentially equal (Table 1, line 1). Therefore, in the experiments described below, one gastrocnemius from each frog served as the test muscle and the other as the control.

When the motor nerve supplying a test muscle was stimulated for 5, 15 or 30 s, in the conditions described in the legend to Table 1, both a maintained tetanic contracture and an increase in the level of muscle cyclic GMP resulted. The increase seemed to be maximal after 15 s, at which time test muscle cyclic GMP concentration was 230% that of the paired control muscle. The effect of stimulation for periods longer than 30 s was not determined because the test muscle fatigued rapidly beyond this point. In agreement with other reports¹⁰, nerve stimulation did not significantly increase the level of cyclic AMP in frog skeletal muscle.

Table 1 Effect of nerve stimulation on cyclic nucleotide levels in frog skeletal muscle

Duration of stimulation (s)	Cyclic GMP % control	Cyclic AMP
Unstimulated	101 ± 7 (9)	105 ± 8 (13)
5	162 ± 21 (7)*	129 ± 20 (7)
15	230 ± 45 (19)†	117 ± 14 (14)
30	192 ± 23 (7)‡	117 ± 13 (6)

Paired gastrocnemii, with nerve supply intact, were removed from each frog and preincubated for 1 h at room temperature in frog saline of the following composition: 111 mM Na⁺, 3 mM K⁺, 1.8 mM Ca²⁺, 117.6 mM Cl⁻, 5 mM MOPS (3-(N-morpholino)propanesulphonic acid), adjusted to pH 7.4 with NaOH. One gastrocnemius from each frog was arbitrarily chosen as the test muscle and the other as the control muscle. After the preincubation period, the motor nerve supplying the test muscle was stimulated by a suction electrode with supramaximal pulses of 0.2 ms duration delivered at 30 Hz for 5, 15 or 30 s. Following this stimulation, the control and test muscles were rapidly homogenised in 4.0–7.0 ml of ice-cold 7% trichloroacetic acid with an Ultraturrax. The homogenates were then centrifuged at 27,000 g for 5 min. The protein content of the pellets was determined by the method of Lowry *et al.*¹⁸. 3.0–3.5-ml aliquots of the supernatants were extracted five times with 10 ml of ether, lyophilised and reconstituted in 0.5 ml of 50 mM sodium acetate–2 mM EDTA (pH 6.2). The cyclic GMP content of the reconstituted samples was measured by the radioimmunoassay method of Steiner *et al.*¹⁹, and the cyclic AMP content by the method of Brown *et al.*²⁰. The recovery of exogenous cyclic GMP and cyclic AMP, added to whole muscle homogenates, was 83% and 89%, respectively. When sodium acetate-reconstituted samples were treated with beef heart phosphodiesterase (Boehringer), the cyclic nucleotide content was reduced to undetectable levels. The cyclic GMP and cyclic AMP concentrations of resting muscles were 0.056 ± 0.006 (mean ± s.e.m.; 28 muscles) and 1.13 ± 0.07 (mean ± s.e.m.; 26 muscles) pmol per mg protein, respectively. The protein levels of the two muscles from a given frog were essentially equal; test muscle protein was 102 ± 2% (mean ± s.e.m.; 22 muscle pairs) that of control muscle. For each muscle pair, results were calculated as (100 (cyclic nucleotide content of test muscle in pmol per muscle)/(cyclic nucleotide content of control muscle in pmol per muscle)). The values represent the mean ± s.e.m. of these data; the numbers in parentheses indicate the number of muscle pairs used in each experiment. Statistical significance was calculated using a two-tailed Student's *t*-test.

* Significantly different from unstimulated, $P < 0.05$.

† $P < 0.02$.

‡ $P < 0.01$.

Table 2 shows the effect of nicotinic and muscarinic cholinergic antagonists on the response of cyclic nucleotides to nerve stimulation. Muscle pairs were preincubated for 1 h in frog saline containing the antagonists, and the motor nerve of the test muscle then stimulated for 15 s. Both nicotinic antagonists, curare (5×10^{-4} M) and α -bungarotoxin (10^{-6} M), blocked the nerve-evoked contraction almost completely, and both compounds abolished the elevation of cyclic GMP in response to nerve stimulation (Table 2, lines 3, 4). The muscarinic antagonist scopolamine (5×10^{-4} M) did not visibly affect the nerve-evoked contraction; nerve stimulation in the presence of scopolamine increased the cyclic GMP level of the test muscle to 190% that of the paired control muscle (Table 2, line 5). The difference between the nerve-stimulated increase in cyclic GMP in the presence and in the absence of scopolamine was not statistically significant (compare lines 2 and 5, Table 2; $P > 0.1$ by Student's *t*-test). These data indicate that the nerve-stimulated increase in cyclic GMP resulted from the activation, by endogenous acetylcholine, of nicotinic, rather than muscarinic, receptors.

To determine whether, as in barnacle and smooth muscle, membrane depolarisation, without neurotransmitter receptor activation, is able to increase cyclic GMP in frog skeletal muscle, muscle pairs were preincubated in frog saline for 1 h after which the test muscle was caused to contract by direct electrical stimulation for 15 s (as described in the legend to Table 2). The isometric tension response of the muscle to direct electrical stimulation was similar to that observed during stimulation of the motor nerve. The cyclic GMP content of the directly stimulated test muscle was 227% that of the control muscle (Table 2, line 6). There was also a slight increase in cyclic AMP in these directly stimulated muscles, but this increase was not statistically significant. The cyclic GMP increase elicited in response to direct electrical stimulation was not due to activation of nicotinic receptors by endogenous acetylcholine released from motor nerve terminals, as this increase was not blocked by the nicotinic antagonist α -bungarotoxin. Thus, a 1-h preincubation in 10^{-6} M α -bungarotoxin, which inhibited neuromuscular transmission, did not visibly affect the electrically stimulated contracture, and direct stimulation in the presence of α -bungarotoxin elevated the level of test muscle cyclic GMP to 192% that of the paired control muscle (Table 2, line 7; lines 6 and 7 not statistically different by Student's *t*-test, $P > 0.1$). These data suggest that depolarisation of the muscle membrane in response to activation of acetylcholine receptors, is a step in the sequence of events by which neuromuscular activity elevates the cyclic GMP level of frog skeletal muscle.

Previous studies had demonstrated that activation of muscarinic, but not nicotinic, cholinergic receptors causes an increase in the level of cyclic GMP in many target tissues; the tissues studied included heart^{2,3}, smooth muscle^{3,5}, brain³, salivary gland⁴, sympathetic ganglia⁶ and exocrine pancreas^{7,8}. The present study of frog skeletal muscle represents the first demonstration of a nicotinic cholinergic-mediated increase in cyclic GMP. Previous failures to observe nicotinic-stimulated increases in cyclic GMP may be explained partly by the fact that many of the tissues examined lack nicotinic receptors and partly by the fact that bath-applied agonists rapidly desensitise the nicotinic, as opposed to the muscarinic, receptor (for a review, see ref. 11).

The present results on vertebrate skeletal muscle and previous studies on invertebrate cross-striated muscle⁹ and vertebrate smooth muscle^{4,5} indicate that, in all three types of muscle, contractile activity is associated with an increase in cyclic GMP. In each case, contractile stimuli which depolarise the muscle membrane, without activating neurotransmitter receptors, also increase muscle cyclic GMP levels; these results suggest that the neurotransmitter increases cyclic GMP levels at least in part indirectly through membrane depolarisation, rather than through direct activation of a neurotransmitter-sensitive guanylate cyclase. Other evidence, obtained in barnacle muscle⁹ and in smooth muscle^{4,5}, suggests that the mechanism by which

Table 2 Effect of cholinergic antagonists on cyclic nucleotide levels in stimulated frog skeletal muscle

Preincubation solution	Mode of stimulation of test muscle	Cyclic GMP % control	Cyclic AMP % control
1. Normal saline	None	101 ± 7 (9)	105 ± 8 (13)
2. Normal saline	Nerve stimulation	230 ± 45 (19)*	117 ± 14 (14)
3. 5×10^{-4} M curare-saline	Nerve stimulation	104 ± 13 (11)	97 ± 10 (6)
4. 10^{-6} M α -bungarotoxin-saline	Nerve stimulation	101 ± 16 (5)	112 ± 14 (5)
5. 5×10^{-4} M scopolamine-saline	Nerve stimulation	190 ± 14 (8)†	118 ± 12 (8)
6. Normal saline	Direct stimulation	227 ± 22 (22)†	138 ± 17 (10)
7. 10^{-6} M α -bungarotoxin-saline	Direct stimulation	192 ± 29 (10)*	111 ± 11 (10)

Paired gastrocnemii, with nerve supply intact, were removed and preincubated for 1 h at room temperature in the indicated solutions. 'Nerve stimulation' indicates that the nerves supplying the test muscles were stimulated for 15 s as described in the legend to Table 1. 'Direct stimulation' indicates that test muscles were placed in a plexiglass chamber and a baffle was fitted around the muscles to divide the chamber into two compartments; supramaximal pulses (1-ms duration; 30 Hz) were applied for 15 s between the two compartments. The values represent the cyclic nucleotide content of the test muscle as the mean $\pm$ s.e.m. of that of the paired control muscle. The numbers in parentheses indicate the number of muscle pairs used in each experiment. Statistical significance was calculated using a two-tailed Student's *t*-test.

* Significantly different from unstimulated (line 1); $P < 0.02$.

† $P < 0.01$.

depolarisation increases cyclic GMP involves an increase in the intracellular concentration of free calcium. A similar mechanism may be responsible for the cyclic GMP increase in frog skeletal muscle.

The role of cyclic GMP in muscle function remains unknown. Ong and Steiner¹², using an immunocytochemical procedure, showed that cyclic GMP is predominantly located over the A bands in unstimulated mammalian skeletal muscle, but the functional significance of this observation has not yet been determined. Cyclic GMP may be part of a positive or negative feedback system for regulating the concentration of free calcium in muscle^{13,14}. Another possibility is that cyclic GMP mediates some of the effects of contractile activity on muscle physiology. For example, contractile activity increases the glucose permeability of vertebrate skeletal muscle¹⁵, and can both maintain muscle mass¹⁶ and prevent the appearance of extra-junctional acetylcholine receptors¹⁷ in denervated mammalian muscle. It is possible that cyclic GMP is the intracellular mediator of one or more of these effects. The observation that contractile stimuli elevate cyclic GMP in several types of muscle indicates that this cyclic GMP increase is of physiological importance.

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Effect of guanine nucleotides on striatal dopamine receptors

GUANINE NUCLEOTIDES have been shown to regulate the sensitivity of adenylate cyclase to hormones in several systems¹⁻⁵, including the dopamine-sensitive adenylate cyclase in the caudate nucleus^{2,4}. It has also been reported that in the presence of GTP the affinities of glucagon receptors⁶, β -adrenergic receptors^{7,8}, prostaglandin E_1 receptors⁹ and opiate receptors¹⁰ for agonists are decreased. For β -adrenergic receptors this effect of GTP has been described as 'agonist-specific', as it is seen with agonists but not with antagonists^{7,8}. We report here a similar effect of GTP on the ability of dopamine-receptor agonists to compete for ³H-spiroperidol binding sites on rat striatal membranes. The presence of 0.3 mM GTP led to a four-to-fivefold increase in the K_d values for the inhibition of ³H-spiroperidol binding by dopamine-receptor agonists. No changes in K_d values were observed for antagonists. Dopamine-receptor agonists and antagonists have been defined by the stimulation or inhibition of dopamine-sensitive adenylate cyclase in the neostriatum^{11,12} or by their effects on the canine renal artery¹³. Controversy exists, however, as to whether binding studies using labelled neuroleptics such as spiroperidol actually measure dopamine receptors. Our finding of an agonist-specific effect of GTP supports the conclusion that ³H-spiroperidol is binding to functional dopamine receptors in the caudate nucleus.

The binding of the potent dopamine receptor antagonist ³H-spiroperidol was studied *in vitro* using a direct filtration binding assay (see legend to Fig. 1a and refs 14-16). At 37°C ³H-spiroperidol bound to rat striatal membranes with an observed affinity of approximately 80 pM, reached equilibrium levels in 8 min, and had a half-time of dissociation of 20 min. Specific binding was defined as the difference between ³H-spiroperidol bound in the absence and presence of 2 μ M (+)butaclamol; this constituted 90-95% of total binding. At the same concentration, (-)butaclamol was inactive. GTP had no effect on the binding characteristics of ³H-spiroperidol. Scatchard analysis was consistent with the existence of only a single

class of high-affinity binding site. The presence of GTP did not lead to changes in either the K_d (78 ± 6 pM and 85 ± 5 pM in the absence, and presence of GTP, respectively; $n = 6$) or the density of binding sites for ^3H -spiroperidol (305 ± 1 fmol per mg and 306 ± 1 fmol per mg in the absence and presence of GTP, respectively). The control K_d value in this study was approximately fourfold lower than that reported previously^{14,15}. This is probably due to species differences—Creese *et al.* used bovine caudate¹⁴—and to the use of different tissues—Fields *et al.* used whole rat brain¹⁵.

The addition of GTP led to a decrease in the apparent affinity of dopamine for the ^3H -spiroperidol binding site ($\text{EC}_{50} = 0.003$ mM GTP). The effect of GTP on rat striatal membranes was maximal at the concentration used (0.3 mM GTP). At this high concentration the order of efficacy of purine nucleotides was as follows: $\text{GTP} = \text{GDP} > \text{ITP} \gg \text{GMP} = \text{guanosine} = \text{ATP} = \text{control}$ (Fig. 1a). These results are similar to the effects of purine nucleotides on β -adrenergic receptors⁷.

A variety of dopamine receptor agonists and antagonists effectively competed with ^3H -spiroperidol for binding sites on

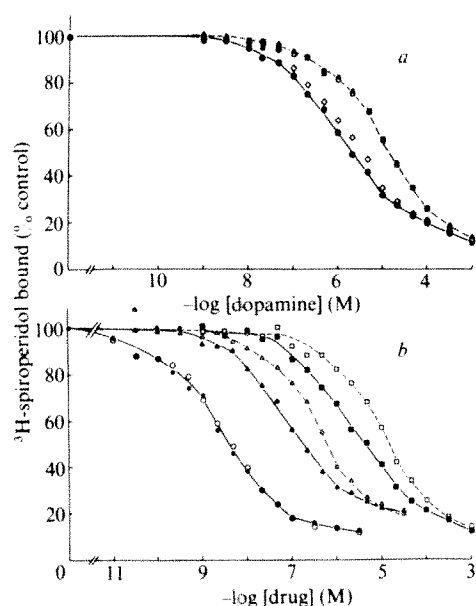


Fig. 1 a, Effect of guanine nucleotides on the inhibition of ^3H -spiroperidol binding by dopamine in rat striatal membranes. Striatal membranes were isolated following homogenisation in 200 volumes of 0.32 M sucrose/10 mM Tris buffer (pH 7.5) by centrifugation at 20,000 g for 10 min at 4°C. Radioligand, drugs and nucleotides were dissolved in 2.8 mM ascorbic acid containing 10 μg per ml bovine serum albumin (BSA). An aliquot of the resuspended membranes (0.1 mg protein in 850 μl of 0.9% NaCl/10 mM Tris buffer, pH 7.5) were added to tubes containing 50 μl ^3H -spiroperidol (26 Ci mmol^{-1} ; 0.4 nM final concentration), 50 μl drug and 50 μl ascorbate/BSA or nucleotide (0.3 mM final concentration). Samples were incubated at 37°C for 10 min; the reaction was then terminated by the addition of 10 ml ice-cold buffered saline and samples were immediately filtered through type-AE glass fibre filters. Each filter was washed at room temperature with an additional 10 ml of buffered saline, and radioactivity was determined by liquid scintillation spectrometry in 7 ml of a Triton X-100/toluene-based fluor. The counting efficiency for tritium was approximately 32%. Points on the displacement curves represent the mean values of three independent determinations. In these experiments ^3H -spiroperidol binding ranged from 800 c.p.m., in the absence of dopamine, to 80 c.p.m., in the presence of 1 mM dopamine. Standard errors (not shown) ranged over 5–15%. ●, Control; ▲, GTP; □, GDP; ◇, GMP. b, The effect of 0.3 mM GTP on the inhibition of ^3H -spiroperidol binding by dopamine-receptor agonists and antagonists in rat striatal membranes. Methods used were identical to those described above. ●, α -flupenthixol; ○, α -flupenthixol + GTP; ▲, $(\pm)$ ADTN; △, $(\pm)$ ADTN + GTP; ■, epinine; □, epinine + GTP.

striatal membranes (Fig. 1b and Table 1). Addition of 0.3 mM GTP to the incubation medium did not affect the inhibition of ^3H -spiroperidol binding by the potent antagonist α -flupenthixol. In the presence of GTP, however, the agonists $(\pm)$ amino-6,7-dihydroxy-1,2,3,4-tetrahydronaphthalene (ADTN) and epinine were less potent in competing for ^3H -spiroperidol binding sites (Fig. 1b). Several dopamine-receptor agonists and the partial agonist apomorphine were less potent in displacing ^3H -spiroperidol binding in the presence of GTP, whereas antagonists were not affected by the presence of GTP (Table 1). The magnitude of the effect was independent of the potency of the agonist tested. Hill coefficients for the agonists/partial agonist were less than unity (0.49–0.61; Table 1) and increased only slightly on the addition of GTP (0.59–0.76). This finding differs from that with β -adrenergic receptors, in which GTP leads to a marked increase in the Hill coefficients for agonists towards unity. In the case of β -adrenergic receptors, Hill coefficients for antagonists are approximately 1.0 in either the presence or absence of GTP⁵.

It should be pointed out that the active isomers of the dopamine-receptor antagonists have Hill coefficients of approximately 0.65, whereas the inactive isomers have Hill coefficients of about 1.0 (Table 1). This difference suggests that the effect of the less active isomers is a reflection of their intrinsic activity and not of contamination with a small amount of the active isomers. It is likely that there are differences in the site or sites occupied by the active and inactive isomers. The Hill coefficients of the antagonists were not changed by the addition of GTP. Low Hill coefficients (<1) could indicate negative cooperativity between individual receptor sites or the existence of multiple binding sites, although it is not known whether either of these explanations is correct.

A possible explanation for the agonist-specific effect of GTP comes from studies of the binding of labelled agonists to glucagon receptors^{1,6}, β -adrenergic receptors⁸, prostaglandin E_1 receptors⁹ and opiate receptors¹⁰. In these systems, guanine nucleotides increase the rate of dissociation of agonists from their binding sites. Agonists, therefore, seem to be less potent in the presence of GTP. Labelled agonists for dopamine receptors have been used in direct binding studies^{17–19}. However, the specific binding of ^3H -dopamine to rat striatal membranes is usually only about 20% of total binding¹⁸. We have thus far been unable to examine the effects of GTP on ^3H -dopamine binding in the rat striatum.

Several reports have suggested that the action of GTP may be involved in the coupling of the receptor with adenylate cyclase^{1,5,20,21}. GTP is required for dopamine stimulation of adenylate cyclase in membranes prepared from homogenates of rat striatum^{2,4}. It is interesting that the hormonal sensitivity of striatal adenylate cyclase, uncoupled from its receptor by exposure to the venom of Russell's viper, was restored by the addition of GTP to the incubation medium²¹. The most direct evidence that the effect of GTP is related to the coupling of receptors with adenylate cyclase has come from studies of the β -adrenergic receptor–adenylate cyclase system in S49 lymphoma cells. In wild-type S49 cells, guanine nucleotides increase the K_d values and Hill coefficients for agonist, but not antagonist, inhibition of ^{125}I -iodohydroxybenzylpindolol binding²⁰. In contrast, S49 cells, in which the β -adrenergic receptor and adenylate cyclase are both present but 'uncoupled', lack an agonist-specific response to GTP. So far, only receptors that can be shown to be linked to adenylate cyclase have been reported to show decreased affinity for agonists in the presence of guanine nucleotides^{1,5–9}. Opiate receptors, which seem to be linked to adenylate cyclase in some systems²², have been reported to show nucleotide regulation of both agonist and antagonist binding¹⁰.

The relationship of neuroleptic binding sites to dopamine receptors has been questioned. The affinities of dopamine-receptor agonists and antagonists differ depending on whether they are determined in the presence of an agonist or antagonist ligand²³. Discrepancies also exist between the agonist and antagonist potencies determined by binding studies when

Table 1 Effect of GTP on the affinity and cooperativity of agonist and antagonist inhibition of ^3H -spiroperidol binding to rat striatal membrane

	Control	K_d (nM) GTP	Ratio	Hill coefficient Control	GTP
Agonists					
Complete					
Epinephrine	390 $\pm$ 100	1400 $\pm$ 200*	3.6	0.56 $\pm$ 0.05	0.61 $\pm$ 0.02
Dopamine	380 $\pm$ 80	1800 $\pm$ 300*	4.7	0.54 $\pm$ 0.02	0.59 $\pm$ 0.03
($\pm$)ADTN	14 $\pm$ 3	54 $\pm$ 6*	3.9	0.49 $\pm$ 0.07	0.59 $\pm$ 0.07
Partial					
Apomorphine	25 $\pm$ 4	90 $\pm$ 29*	3.6	0.61 $\pm$ 0.04	0.76 $\pm$ 0.06*
Antagonists					
(+)-Butaclamol	0.61 $\pm$ 0.10	0.67 $\pm$ 0.19	1.1	0.65 $\pm$ 0.03	0.66 $\pm$ 0.04
(-)-Butaclamol	3400 $\pm$ 400	3900 $\pm$ 600	1.1	0.97 $\pm$ 0.01	0.94 $\pm$ 0.05
α -Flupenthixol	0.37 $\pm$ 0.04	0.45 $\pm$ 0.13	1.2	0.65 $\pm$ 0.07	0.62 $\pm$ 0.06
β -Flupenthixol	52 $\pm$ 11	52 $\pm$ 4	1.0	1.02 $\pm$ 0.06	0.96 $\pm$ 0.04

Assays were carried out as described in the legend to Fig. 1a. Each ratio of K_d values was calculated as the K_d in the presence of GTP to the K_d in the absence of GTP. The concentration of GTP was 0.3 mM. The results are expressed as the mean $\pm$ s.e.m. calculated from three to four independent determinations.

* Means values significantly different; Student's *t* test, $P < 0.05$.

compared with those determined in studies of dopamine-stimulated adenylate cyclase activity^{13,23}. Further, the subcellular distributions of ^3H -spiroperidol binding sites and adenylate cyclase in the rat brain are not identical²⁴. From such studies, it has been suggested that the dopamine receptor and adenylate cyclase are related but not identical sites. Also, decreases in dopamine-sensitive adenylate cyclase activity (85%) and ^3H -haloperidol binding (40%) occur following intrastriatal injection of kainic acid²⁵. The difference in the magnitude of the effects of kainic acid has led some investigators to question whether striatal adenylate cyclase and ^3H -haloperidol binding sites are associated. The data presented here demonstrate a specific effect of GTP on the inhibition of ^3H -spiroperidol binding by the same agonists which have previously been shown to activate adenylate cyclase^{11,12}. The connection between the striatal ^3H -spiroperidol binding site and the dopamine-sensitive adenylate cyclase is thus strengthened^{15,16}.

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Mammary tumour virus and casein gene transcription during mouse mammary development

MORPHOGENESIS and differentiation of the murine mammary gland is dependent on the interaction of peptide and steroid hormones. These hormones cause mammary growth through proliferation of ductal and alveolar epithelial cells^{1,2} and differentiation of the gland for the production of the milk proteins, casein and α -lactalbumin^{3,4}. Mammary development frequently increases the incidence and results in an earlier onset of mammary tumours^{5,6}. The influence of mammary development upon tumorigenesis has been correlated with the appearance of mouse mammary tumour virus (MMTV) particles⁶⁻¹⁰. Importantly, the production of MMTV particles is dependent on alveolar differentiation and function^{7,11}. Lactating mammary glands from BALB/c mice contained considerably lower levels of MMTV RNA than glands from C3H mice infected with the milk-transmitted virus MMTV-S (ref. 12). In this study we have examined the influence of the hormonal changes that occur during mammary development on MMTV and casein expression by molecular hybridisation with specific complementary DNA (cDNA) probes to MMTV 70S RNA and casein 15S mRNA. Hence, the relationship between MMTV expression and the expression of a normal differentiated mammary function—the production of casein mRNA—was determined. We have quantitated MMTV RNA and casein mRNA in developing mammary tissues from BALB/c and BALB/c/c3H mice that have a low and high incidence of mammary tumours respectively^{6,13}. These studies demonstrate that the hormonal alterations occurring during mammary development enhanced MMTV RNA expression to a greater extent in the high than the low tumour-incidence strain, whereas similar changes in the level of casein mRNA were observed in both strains during mammary development.

A representative MMTV cDNA probe was synthesised *in vitro* by avian myeloblastosis virus reverse transcriptase using 70S MMTV RNA as template and calf thymus DNA fragments¹⁴ as a random primer^{15,16}. The level of MMTV RNA was quantitated in cellular RNA extracts by the titration method¹⁷. The amount of MMTV RNA, expressed as a percentage of the

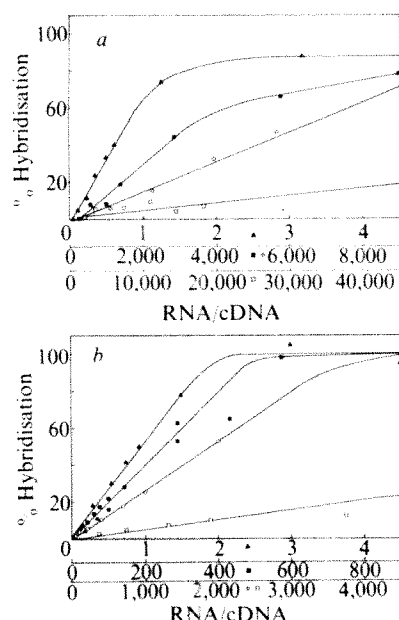


Fig. 1 Detection of MMTV RNA and mouse casein 15S mRNA in mammary glands by molecular hybridisation. Cellular RNA was isolated by sodium dodecyl sulphate-phenol extraction with proteinase K digestion²⁰ followed by extraction with 3 M sodium acetate²¹. Hybridisations were carried out and assayed by S1 nuclease digestion¹⁹. *a*, Hybridisations of MMTVcDNA, equivalent Dot of 0.09, with MMTV 70S RNA isolated from purified MMTV virions ($\blacktriangle$) and RNA isolated from early pregnant ($\square$), late pregnant ($\blacksquare$) and late lactating ($\circ$) uniparous BALB/cfC3H mammary tissues. *b*, Hybridisations of mouse casein cDNA, equivalent Dot of 0.09, with purified mouse casein 15S mRNA ($\blacktriangle$) and RNA isolated from early ($\square$), mid ($\circ$) and late ($\blacksquare$) pregnant uniparous BALB/cfC3H mammary tissues.

cellular RNA, was determined by comparing the rate of hybridisation observed with each cellular RNA to the rate of hybridisation observed with purified 70S MMTV RNA (Fig. 1*a*). Casein mRNA was quantitated in the same manner (Fig. 1*b*) with a full-length cDNA probe synthesised from purified mouse casein 15S mRNA¹⁸ as described previously¹⁹. The limit of detection of MMTV RNA and casein mRNA was 0.0005% of the total cellular RNA.

Casein mRNA was quantitated in mammary tissues from virgin and uniparous BALB/c and BALB/cfC3H mice at various stages of mammary development. Figure 2*a* shows that the level of casein mRNA, which was at a very low level in the virgin gland (0.0009%), was present at an appreciable level in early pregnancy (0.0066%) and then increased to 0.365% of the RNA late in pregnancy. Casein mRNA increased nearly four-fold from late pregnancy to early lactation and was maintained at a high level, approximately 1% of the RNA, during lactation. Following weaning, casein mRNA decreased to one tenth or less of the lactating level, but was considerably higher than observed in the virgin gland. Essentially identical changes in the level of casein mRNA were observed during mammary development in both the low and the high mammary tumour incidence strains, BALB/c and BALB/cfC3H, respectively.

MMTV RNA was quantitated in mammary tissues from virgin and uniparous BALB/c and BALB/cfC3H mice at various stages of mammary development (Fig. 2*b*). The increased tumour incidence of BALB/cfC3H mice relative to the isogenic strain, BALB/c, is due to the milk-transmitted virus, MMTV-S, obtained by foster-nursing BALB/c mice on C3H. Note that normal BALB/c and BALB/cfC3H tissues contain 5–9 copies of MMTV DNA per diploid cell²². The level of MMTV RNA in the mammary tissues was below the limit of detection in virgin and early pregnant BALB/cfC3H mammary glands and was present at low levels (0.0014%) in mid pregnancy. MMTV RNA was at an appreciably higher level (0.0125%) in the

BALB/cfC3H mammary gland at late pregnancy and increased approximately twofold after parturition. Later in lactation, the level of MMTV RNA decreased from the level observed in early lactating BALB/cfC3H mammary glands. The decrease in MMTV RNA late in lactation may be due to the release of MMTV RNA particles into glandular secretions^{6–8,10,11}. MMTV RNA levels were similar in BALB/cfC3H mammary glands late in lactation and 10 days after weaning, 0.0076% and 0.0096%, respectively. Large variations in the amount of MMTV RNA (Fig. 2*b*) but not casein mRNA (Fig. 2*a*) were observed in pregnant and lactating BALB/cfC3H mammary tissues. Interestingly, differences in milk virus titre, particularly in uniparous mice, have been reported to be indicative of similar biological variation in virus production⁸. The level of MMTV RNA in BALB/c mammary tissues at all the developmental stages assayed was 0.0005% or less.

The hormonal changes that control normal mammary development also influence neoplastic development of the mammary gland^{5,6}. In BALB/c and BALB/cfC3H mice the mammary tumour incidence is higher in retired breeders than virgin mice (refs 6, 13 and D. Medina, personal communication). The occurrence of MMTV antigens in milk from lactating mice increases with the number of lactations in certain mouse strains^{9,10,23}. Mammary tumours from BALB/c and BALB/cfC3H retired breeders have higher levels of MMTV RNA than observed in uniparous lactating mammary glands (R. J. P. and S. H. S., in preparation). These observations suggest that additional cycles of hormonal stimulation during pregnancy and lactation may increase the level of MMTV RNA in mammary glands. Therefore, the level of MMTV RNA was quantitated in mammary glands from BALB/c and BALB/cfC3H lactating multiparous mice and retired breeders (Table 1). The levels of casein mRNA in multiparous lactating mice and retired

Fig. 2 Mouse casein 15S mRNA and MMTV RNA in mammary tissues during mammary development. Tissues were obtained from BALB/c and BALB/cfC3H virgin mice (Vi) and either pregnant (Pg), lactating (La) or post-weaning (PW) uniparous mice. Early (E), mid (M) and late (L) pregnant mice were staged at 1–7, 8–14, and 15–21 days of pregnancy, respectively. Early (E), mid (M) and late (L) lactating mice were staged at 1–7, 8–14 and 15–21 days of lactation respectively. Post-weaning mammary glands were obtained from uniparous mice 5 or 10 days following weaning. Cellular RNA was prepared and hybridisations were carried out as described in the legend to Fig. 1. The amount of casein mRNA (Fig. 2*a*) and MMTV RNA (Fig. 2*b*) are expressed as a percentage of the cellular RNA. Solid bars denote BALB/c tissues and cross-hatched bars denote BALB/cfC3H tissues, presented as the mean $\pm$ one s.e.m. for (*n*) determinations.

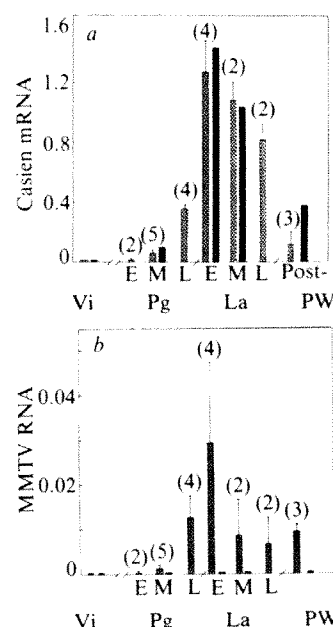


Table 1 MMTV RNA and casein mRNA in mammary tissue from multiparous mice and retired breeders

Strain	Developmental stage	MMTV RNA (% total RNA)	Casein mRNA (% total RNA)
BALB/cfC3H	Lactating-multiparous	0.0181	1.700
BALB/cfC3H	Retired breeder	0.0128	0.017
BALB/c	Lactating-multiparous	0.0006	1.790
BALB/c	Retired breeder	0.0009	0.014

Mammary tissue excised from 5–7 day lactating multiparous mice and retired breeders, obtained one month after weaning of the fourth litter, were used for the assays. Cellular RNA was prepared and hybridisations were performed as described in the legend to Fig. 1.

breeders of both strains were consistent with the developmental stage of the gland. In BALB/cfC3H mammary glands from multiparous lactating mice and retired breeders (Table 1), the level of MMTV RNA was similar to that observed in uniparous lactating, 0.0295%, and weaned, 0.0096%, mice. Mammary tissues from BALB/c retired breeders contained a low but detectable amount of MMTV RNA (0.0009%) whereas uniparous and multiparous BALB/c lactating mammary tissues contained lower levels of MMTV RNA ($\leq 0.0006\%$). Varmus *et al.*¹² also have reported that lactating BALB/c mammary glands contained low levels of MMTV RNA (0.0003%). Therefore, additional cycles of hormonal stimulation increased MMTV expression in BALB/c but not BALB/cfC3H mammary glands.

These studies indicate that the hormonal milieu required for mammary development and lactogenesis enhanced both casein and MMTV expression in the mammary gland of the high tumour incidence strain, BALB/cfC3H. Both MMTV RNA and casein mRNA were detectable by mid pregnancy and increased until the maximum level was observed early in lactation. Although the level of both MMTV RNA and casein mRNA decreased following weaning in both uniparous and multiparous mice, MMTV RNA did not decrease to the same degree as casein mRNA. Therefore, the accumulation of MMTV RNA in BALB/cfC3H mammary glands is dependent upon mammary development and influenced by differentiated mammary function. Consequently, the accumulation of B-type virus particles, which is dependent upon alveolar differentiation and function^{8,11}, is also related to and may be dependent upon accumulation of MMTV RNA in the mammary gland of high tumour incidence strains. Interestingly, an appreciable level of MMTV RNA was maintained in the absence of continued hormonal stimulation of the BALB/cfC3H gland. Casein expression in the low tumour incidence strain, BALB/c, was influenced similarly by the hormonal changes that occur during mammary development. However, MMTV expression during mammary development in BALB/c mice was not influenced to the same extent as observed in BALB/cfC3H mice. Therefore, MMTV expression in the low tumour incidence strain is apparently not influenced by hormonally-regulated mammary development to the same degree as in the high tumour incidence strain.

Genetic and biochemical evidence indicate that mouse strains contain different variants of MMTV^{6,24–28}. MMTV-O is apparently an endogenous virus transmitted as a germinal provirus in BALB/c mice^{24,25}, while BALB/cfC3H mice contain both MMTV-O and the exogenous virus, MMTV-S, transmitted by foster-nursing. Our studies indicated that MMTV-O and MMTV-S do not respond to the same extent to changes occurring in the hormonal environment during normal mammary development. Further studies using specific nucleic acid probes²⁶ or antibodies to type-specific antigenic determinants^{27,28} should provide additional evidence that MMTV-O and MMTV-S respond differently to changes in the hormonal environment during normal mammary development. The effect of mammary development on MMTV expression is related to

the tumour incidence of the strain, suggesting that multiple factors may influence MMTV expression during normal development.

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Male recombination is not induced in *Drosophila melanogaster* by extracts of strains with male recombination potential

NON-TRIVIAL frequencies of spontaneous male recombination in the hybrid progeny of *Drosophila melanogaster* males from so-called 'male recombination strains' and laboratory marker stock females are well documented^{1,2}. Attempts to induce male recombination by injection have produced conflicting results. The positive results originally claimed by Reddi *et al.*³ were not reproducible in two subsequent studies^{4,5}. Sochacka and Woodruff⁶ have suggested that the disparity in results may be attributable to the use of extracts from a male recombination strain in the former study only. They injected extracts from the male recombination strain OK1 (ref. 7) into laboratory marker strain females. These were crossed to the Canton-S strain, which lacks spontaneous male recombination potential, producing F₁ hybrids which yielded a low frequency of male recombination. Injection directly into F₁ hybrid males also yielded some recombinants. Similar tests using Canton-S as the source of the extract were negative. We describe here large-scale tests with different male recombination strains. We could not reproduce the injection effect of Sochacka and Woodruff, suggesting that their results may not be generally applicable. We suggest, furthermore, that the genetic implications of a negative injection result may be at least as interesting as those of a positive result. A positive result would imply the existence of some co-existing,

Table 1 Observed numbers of recombinants and the minimum number of recombination events in various studies

	Experimental		Total	Control		Total
	Recombinants	Minimum events		Recombinants	Minimum events	
	Actual			Actual		
Sydney	7	6	116,776	9	6	70,548
Brown	9	6	39,628	22	15	86,024
Sochacka and Woodruff ⁶	25	11	23,170	0	0	67,910

'Experimental' includes all cases of injections of extracts from male recombination strains into females and males.
'Control' includes injection with non-recombination strains, injection of solvents and no injection.

possibly non-essential, factor, such as a virus or episome. This would rule out explanations for male recombination based on incompatibility of genetic organisation in different strains. Explanations of this type, for example, in terms of spatial organisation of chromosomes⁸, have potentially important implications for normal nuclear organisation.

The experiments were carried out independently at Sydney University and at Brown University. Extracts from the strong male recombination strains, Harwich² and Hunter Valley⁸, were used in the experimental groups and extracts from the laboratory strain Canton-S were used as controls in both laboratories. In Sydney, Harwich or Hunter Valley extracts were injected into females of the laboratory strain *al cn bw* (chromosome II). At Brown, Harwich extracts were injected into the laboratory strains *al cl b c sp²* (chromosome II) and *rucuca* (chromosome III). The majority of control flies were injected with Canton-S extracts, a few were injected with Ringer's solution and a few were untreated. Experimental and control tubes were coded and combined for counting, an important consideration where low recombination frequencies are expected.

Altogether, 10 experiments were carried out. The initial experiments were made using the concentration of extract given by Sochacka and Woodruff⁶. Subsequently, concentrations up to four times higher were used. We also examined the effect of ageing the injected flies, in case a specific developmental stage or incubation period was important. In addition, following a suggestion by Dr D. Angus of Newcastle University, New South Wales, we injected extracts from various hybrids rather than from pure strains, on the grounds that male recombination might be attributable to an agent which multiplies preferentially in the hybrid.

None of the experiments showed an elevated level of recombination. Results have therefore been pooled within laboratories, and are presented in the first two lines of Table 1. We show both the actual number of recombinants found and the minimum number of independent recombination events⁹. Neither of these numbers is an entirely satisfactory measure of recombination intensity. The actual number of recombinants cannot validly be used in statistical tests if recombinants occur in clusters rather than independently of each other. On the other hand, comparisons based on the minimum number of recombination events are strictly valid only if a fixed number of progeny is screened per cross. In the present experiments, however, neither measure indicates an effect of the treatment.

The data of Sochacka and Woodruff⁶ are summarised in the third line of Table 1. The complete absence of recombinants in their control series is noteworthy considering that Canton-S was used by all three laboratories. It is a major cause of the significance of the difference in recombination frequency between their experimental and control groups. Also, the frequency in the experimental injected group is about an order of magnitude lower than the spontaneous frequency in OK1 hybrid males⁷.

There are, of course, several possible reasons for dissimilar results using different strain combinations. We suggest, nevertheless, that the hypothesis of direct transmissibility of male recombination by injection requires further substantiation.

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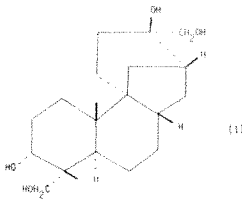
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Aphidicolin prevents mitotic cell division by interfering with the activity of DNA polymerase- α

THE study of the control of DNA synthesis would be helped if a variety of chemicals which influenced DNA synthesis in various ways was available. We have searched for chemicals which prevent mitotic division of sea urchin embryos, which requires DNA synthesis, but do not prevent meiotic divisions of starfish oocytes, which are independent of DNA synthesis¹. Aphidicolin² (I) is one such compound. Furthermore, recent experiments have shown that aphidicolin selectively inhibits the activity of DNA polymerase- α obtained from regenerating rat liver without interfering with the activity of DNA polymerase- β and mitochondrial DNA polymerase³. The function of the DNA polymerases in the complex process of DNA replication is not known. Correlative studies of DNA synthesis and the level of DNA polymerase activity suggest that DNA polymerase- α may be important in DNA replication⁴. However, in certain cases, DNA polymerase- β and - γ also increase at the time of DNA synthesis, and attempts to specify which of the DNA polymerases is 'replicative' or to assign roles to the various polymerases have not yet been successful⁴. We report here evidence that prevention of cell division by aphidicolin in sea urchin embryos is due to selective inhibition of DNA polymerase- α activity. This study implies a functional role for this polymerase activity during replication of DNA.



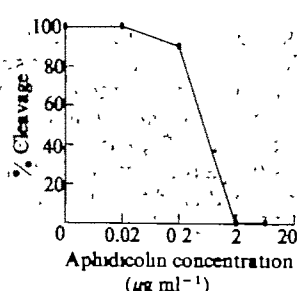


Fig. 1 Inhibition by aphidicolin of mitotic division of embryos of *Hemicentrotus pulcherrimus*. Five min after fertilisation, embryos were treated with various concentrations of aphidicolin. Morphological changes were observed for 150 min. Aphidicolin (insoluble in aqueous media) was dissolved in DMSO and then dispersed in seawater to give a DMSO concentration of 0.1% (v/v) in the solution, which did not affect cleavage.

When embryos of the sea urchin *Hemicentrotus pulcherrimus* were exposed to aphidicolin ($2 \mu\text{g ml}^{-1}$) for 150 min from 5 min after insemination, nearly all of them remained at the 1-cell stage whereas control embryos divided three times (Fig. 1). The incorporation of ^3H -thymidine into DNA of the embryos was inhibited by 90% by treatment with aphidicolin at $2 \mu\text{g ml}^{-1}$ (Table 1). The rate of protein synthesis was the same whether or not embryos were exposed to the compound (Table 1). RNA synthesis remains quite low during early cleavage and maternal messenger RNA synthesised during oogenesis and stored in eggs plays a central role in translation⁵. Therefore, the effect of aphidicolin on RNA synthesis was examined at the gastrula stage. The incorporation of ^{14}C -uridine into the acid-insoluble material was not affected by 1 h exposure to aphidicolin ($10 \mu\text{g ml}^{-1}$).

Aphidicolin ($40 \mu\text{g ml}^{-1}$) did not prevent 1-methyladenine-induced (45 ng ml^{-1}) meiotic maturational divisions in oocytes of the starfish *Asterias amurensis*⁶. Meiosis is a reduction in chromosome number and no chromosomal replication takes place during the maturational divisions. Thus, it seems that aphidicolin prevents the sequence of events necessary to complete chromosomal replication in sea urchin embryos rather than the process of karyokinesis and cytokinesis.

Inhibitors of deoxyribonucleotide biosynthesis such as *N*-hydroxyurea ($200 \mu\text{g ml}^{-1}$) and 5-fluorouracil ($200 \mu\text{g ml}^{-1}$) did not affect the first cleavage of sea urchin embryos. In fact,

Table 1 Effects of aphidicolin on DNA and protein synthesis in embryos of *Hemicentrotus pulcherrimus*

Aphidicolin concentration ($\mu\text{g ml}^{-1}$)	^3H -Thymidine incorporation (%)	^3H -L-Leucine incorporation (%)
0	100	100
0.02	76	105
0.2	38	125
2	10	124
10	5	110

Five min after fertilisation, ^3H -thymidine ($0.63 \mu\text{Ci}$; 24 Ci mmol^{-1}) and various amounts of aphidicolin were added to 5×10^5 eggs in 5 ml of seawater. Ninety min later, embryos were sedimented, washed, fixed and extracted with 1 M perchloric acid, and the acid-insoluble radioactivity was counted according to the method of Hinegardner *et al.*⁷. The radioactivity in the control experiment was 1.7×10^4 c.p.m. ^3H -L-Leucine (0.2 mCi ; 58 Ci mmol^{-1}) was added to 4×10^5 unfertilised eggs suspended in 20 ml of seawater. Five min later, eggs were thoroughly washed with seawater, suspended in 20 ml of seawater, inseminated and various amounts of aphidicolin were added to 2.5-ml aliquots of the suspension. Ninety min later, embryos were collected, fixed and extracted with 10% TCA at 90°C , and the acid-insoluble radioactivity was counted according to the method of Mano⁸. The control value was 5.9×10^3 c.p.m. Aphidicolin was dissolved in DMSO and then dispersed in seawater to give a final concentration of 0.1% DMSO (v/v), which did not affect DNA or protein synthesis in the embryo.

Table 2 Effects of aphidicolin on activity of DNA polymerase- α , - β and - γ obtained from the nuclear fraction of blastulae of *Hemicentrotus pulcherrimus*

Aphidicolin concentration ($\mu\text{g ml}^{-1}$)	DNA polymerase activity (%)		
	α	β	γ
0	100	100	100
0.5	39	—	—
2	10	102	95
10	0	99	98

DNA polymerase- α was purified from the nuclear extract using phase separation and hydroxyapatite and phosphocellulose columns. The properties of the polymerase were similar to those described by Loeb¹⁰. DNA polymerase- β was obtained as described elsewhere¹¹. The activated DNA-dependent DNA synthesis with DNA polymerase- α and - β were determined using 50 mM Tris-maleate buffer, pH 8.5, as described previously¹¹. The poly(rA)-oligo(dT)₁₂₋₁₈ dependent DNA polymerase- β and - γ activities were measured as described elsewhere (Habara, A.; Nagano, H. & Mano, Y., in preparation). In the case of DNA polymerase- β , the values shown in this table were obtained by using activated DNA as template primer. The poly(rA)-oligo(dT)₁₂₋₁₈ dependent DNA polymerase- β activity was also insensitive to aphidicolin. The activity of DNA polymerase- α , - β and - γ in the absence of aphidicolin was 3,200, 600 and 5,200 c.p.m., respectively. Aphidicolin was dissolved in DMSO and then dispersed in the reaction mixtures to give a final DMSO concentration of less than 0.1% (v/v). At these concentrations, DMSO did not affect the activity of the DNA polymerases.

unfertilised sea urchin eggs have enough pools of four deoxyribonucleoside triphosphates to support two or three cycles of DNA replication⁷. This excludes the possibility that aphidicolin acts by inhibiting deoxyribonucleotide biosynthesis, thereby depriving DNA polymerases of necessary substrates.

The effects of aphidicolin on the activity of DNA polymerase- α , - β and - γ obtained from the nuclear fraction of the sea urchin embryos (blastulae) were next examined. The addition of aphidicolin ($2 \mu\text{g ml}^{-1}$) inhibited the activity of DNA polymerase- α by 90% whereas that of DNA polymerase- β and - γ was not affected by the treatment (Table 2). Thus the degree of inhibition by aphidicolin of the activity of DNA polymerase- α was nearly the same as that observed for cleavage and DNA synthesis in the intact cell (Fig. 1, Tables 1 and 2). However, it was observed that a higher dose of aphidicolin was required to inhibit the activity of DNA polymerase- α which was further purified by a DEAE-Sephadex A-50 column; aphidicolin at $2 \mu\text{g ml}^{-1}$ produced 58% inhibition of DNA synthesis with this preparation. This could be due to a change in the enzyme molecule from its native state during purification or could be related to the adherence of some component to the enzyme which facilitates the action of the drug and is separated from the enzyme by DEAE-Sephadex chromatography. We are at present carrying out further studies designed to test these possibilities. Similarly, aphidicolin inhibited the activity of DNA polymerase- α obtained from unfertilised eggs but did not affect DNA polymerase- β activity. These results suggest that DNA polymerase- α plays an essential part in chromosomal DNA replication in cleavage of sea urchin embryos. Thus aphidicolin has considerable potential for investigating the fundamental nature of the cell functions concerned.

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Resistance to viomycin conferred by RNA of either ribosomal subunit

THE basic peptide antibiotic viomycin inhibits prokaryotic protein biosynthesis^{1,2}, and seems to bind at least to the small ribosomal subunit because it competes partially with the binding of streptomycin³. However, viomycin does not induce misreading like streptomycin¹, but promotes association of the ribosomal subunits, stabilises 70S couples⁴, and blocks translocation^{5,6}. Interestingly, resistant mutants have been found containing either an altered 30S or an altered 50S subunit^{7–9}. Viomycin strengthens the association of the sensitive subunits but does not affect the association of subunits derived from resistant mutants⁴. These mutants have been obtained from *Mycobacterium smegmatis*, which is highly sensitive in culture in contrast to *Escherichia coli*. We describe here the formation of totally reconstituted subunits using components of sensitive and resistant ribosomes. The reconstituted particles were tested with their complementary, sensitive subunit with respect to viomycin-induced 70S couple formation. The resistance property of either subunit was shown to be located in the RNA moiety.

In the first series of experiments two Rabinowitchi (R) strains of *M. smegmatis* were used: R15 as the viomycin sensitive parental strain and the resistant mutant R33 which has an altered 30S subunit⁹. Intact 16S RNA from 30S subunits of both strains was isolated by the phenol method¹⁰. The isolation of the total proteins (TP30) and the reconstitution procedure for the small subunit have been reported¹¹. RNA and protein moieties from the sensitive and resistant strains were reconstituted in all combinations. The reconstituted particles were supplemented with sensitive 50S (R15) subunits and analysed on sucrose gradients. The conditions of the sucrose gradient centrifugation were chosen so that sensitive ribosomes only formed appreciable amounts of 70S couples in the presence of the drug⁴. Thus, when the ribosomal component conferring the resistance was used for reconstitution, viomycin should not be able to induce the formation of 70S couples.

Figure 1 shows the results. The homologous reconstitutions behaved as expected. 30S particles reconstituted with components of the sensitive strain form 70S couples in the presence of viomycin (Fig. 1b), whereas those obtained with resistant components do not (Fig. 1d). The heterologous reconstitution shows a sensitive response (70S couple formation) when parental 16S RNA was used (Fig. 1f). In contrast, a resistant response was found in the case where mutant 16S RNA was reconstituted with parental TP30. Thus, the resistance

follows the 16S RNA and is not related to the protein moiety (TP30). We conclude that the resistance of the strain R33, which has an altered 30S subunit, is conferred by the 16S RNA.

A similar analysis was performed with native, sensitive 30S subunits and reconstituted 50S particles. 50S subunits were obtained from R15 as the sensitive strain and from the resistant mutant R31 which has an altered 50S subunit⁹. The procedures for the isolation of (23S + 5S) RNA and total proteins (TP50)

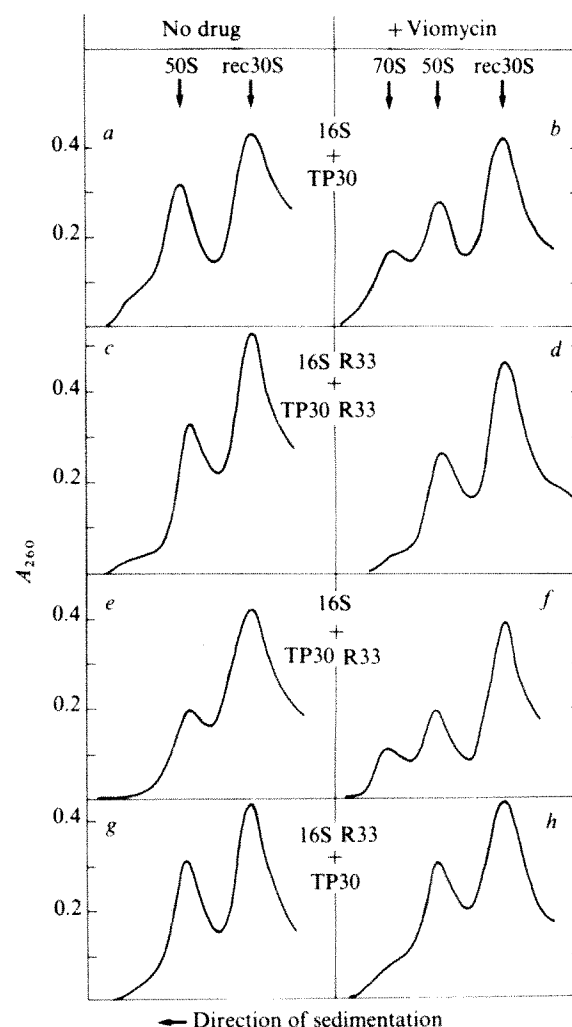


Fig. 1 Sedimentation profiles of reconstituted 30S particles and wild-type 50S subunits in the presence and absence of viomycin. In the insets the ribosomal components are listed which were used for reconstitution of the 30S particle: where not indicated as R33 the 16S RNA (16S) and the proteins (TP30) were derived from 30S subunits of the sensitive parental strain R15. Cells were grown and 70S ribosomes prepared as described previously⁷. To remove attached RNase, ribosomes were washed with a buffer containing 10 mM Tris-HCl, pH 7.5, 0.5 M LiCl and 10 mM Mg acetate (ref. 10). The isolation of subunits and RNA (from the subunits which contain intact RNA) was as described¹⁰, the preparation of total proteins TP30 followed ref. 11. For total reconstitution of 30S particles 2.5 A_{260} units of 16S RNA and 0.4 A_{230} units of TP30 in 20 mM Tris-HCl (pH 7.6), 400 mM NH_4Cl , 20 mM Mg acetate, 0.2 mM EDTA and 2 mM β -mercaptoethanol were incubated in 100 μl at 40° for 20 min. One A_{260} unit of reconstituted 30S particles were mixed with 0.4 A_{260} units of parental 50S subunits in a buffer containing 10 mM Tris-HCl, 4 mM Mg acetate and 60 mM NH_4Cl , and where indicated 15 μM viomycin. The mixture was incubated for 10 min at 37° and loaded on a sucrose gradient (10–30%) with the same ionic conditions and where indicated with 15 μM viomycin. After centrifugation for 19 h at 17,000 r.p.m. and 4°C in a Beckman SW40 rotor the gradients were automatically monitored for A_{260} .

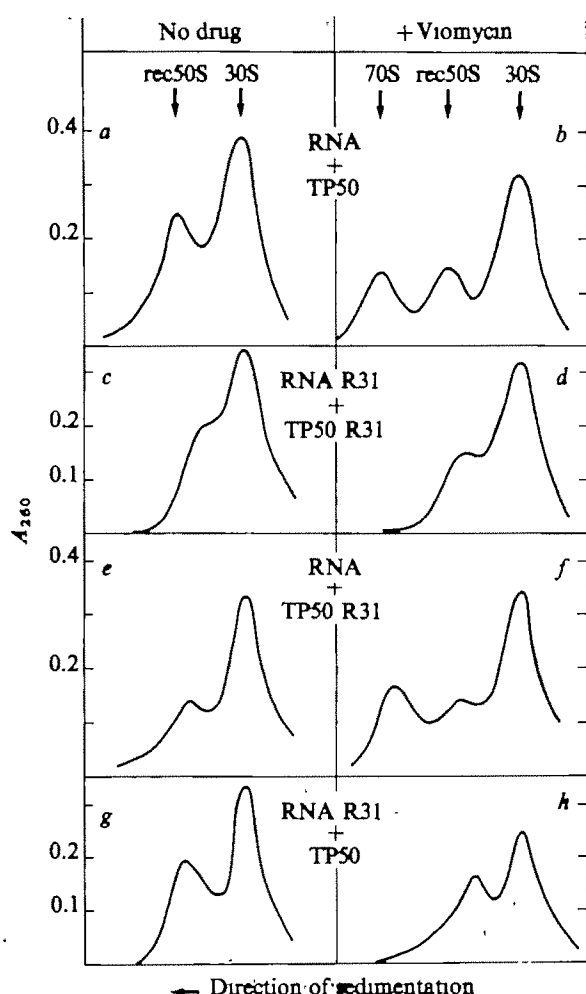


Fig. 2 Sedimentation profiles of wild type 30S subunits and reconstituted 50S particles in the presence and absence of viomycin. Where not indicated as R31 the (23S+5S) RNA (RNA) and the total proteins (TP50) were derived from 50S subunits of the sensitive parental strain R15. Isolation of subunits and RNA was as described in Fig. 1, the preparation of TP50 followed ref. 10. For total reconstitution of 50S particles 2.5 A_{260} units of (23S+5S) RNA and 0.4 A_{260} units of TP50 were incubated in the conditions of the two-step incubation¹⁰. Parental 30S subunits (0.6 A_{260} units) were mixed with 0.4 A_{260} units of reconstituted 50S particles, incubated and analysed on sucrose gradients as described in Fig. 1.

and for the reconstitution of the large subunit have been described¹⁰. Both homologous and heterologous reconstitutions led to particles sedimenting at the 50S position (Fig. 2). In the presence of viomycin we found a pattern equivalent to that shown for reconstituted 30S particles in Fig. 1: All reconstituted 50S particles containing parental RNA react as sensitive particles (70S couple formation, Fig. 2b, f), whereas those containing mutant RNA behave as resistant particles (no 70S couple formation, Fig. 2d, h). We conclude that the resistance of strain R31 which has an altered 50S subunit is also conferred by the RNA moiety of the large subunit.

Both reconstituted 30S and reconstituted 50S particles containing mutant 16S and (23+5S) RNA, respectively, could form 70S couples with their complementary native subunits only in the presence of higher Mg^{2+} concentration (above 12–15 mM; data not shown). In spite of their normal sedimentation and association behaviour the reconstituted particles were only poorly active in *in vitro* systems of protein synthesis.

Viomycin is the fourth example where resistance against an antibiotic is located in the RNA. Kasugamycin-resistant ribo-

somes contain an altered 16S RNA; the RNA is under-methylated near the 3' end¹². Erythromycin resistance can be due to an over-methylated 23S RNA¹³. Recently, a thiostrepton producing organism was described which protects itself against this drug by methylating the RNA from the large ribosomal subunit¹⁴. However, viomycin is the only case so far known in which the resistance against the drug can be located in the RNA moiety of either the small or the large subunit. Taken together with our observation that viomycin induces association these findings suggest that the RNA of both subunits is involved in the association reaction.

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Corrigendum

In the letter 'Visual response in barnacle photoreceptors is not initiated by transitions to and from metarhodopsin' by Z. Atzmon, P. Hillman and S. Hochstein, *Nature* 274, 76 (1978), the words 'blue' and 'red' heading the third and fourth columns of Table 1 should be interchanged.

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matters arising

Relative merits of 'extreme-pressure' additives

THE relative merits of 'extreme-pressure' additives are best examined under comparable conditions of 'mild wear' when only a fine dust is abraded from the rubbing surfaces and no macroscopic damage has been done. Macroscopic damage always leads to 'catastrophic' conditions and must therefore be avoided to ensure safe operation for a practically indefinite period. The criterion for safe operation is the validity of Amontons' law. The principal function of any extreme-pressure additive is to extend substantially the range of normal loads in which one can be certain that only mild wear will take place, so that bearing pressures of at least $15,000 \text{ kg wt cm}^{-2}$ are maintained. An optimum concentration¹ exists for each extreme-pressure additive which can react with the lubricated surfaces. Any comparison of extreme-pressure additives must therefore be made at their optimum concentrations and at normal loads for which Amontons' law is obeyed. With 0.9255-cm diameter En-31 steel balls in a four-ball apparatus running at 1,500 r.p.m. the optimum concentrations of two different chlorinated paraffin waxes and of benzyl chloride in a light mineral oil were respectively 5, 2 and 1% by weight, corresponding to 59, 29 and 8 mg-atoms of chlorine per 100 g of oil².

The existence of an optimum concentration suggests that two counteracting processes are responsible for the action of extreme-pressure additives. It is difficult to see how the formation of a protective coating on its own could account for an optimum concentration, but the view expressed recently by McCarroll *et al.*³ that extreme-pressure lubricants containing either sulphur or chlorine form with steel "surface layers of iron sulphide or chloride which prevent metal wear and seizure" is quite unacceptable, because mild wear always takes place, even if the wear prints have a mirror finish. What the extreme-pressure additive does to extend the range of validity of Amontons' law of its base oil, is to lower both the force of friction and the rate of wear which would be encountered with the base oil. At their optimum concentrations each of the chlorine-containing additives mentioned above reduced the coefficient of friction of the base oil by 15%, from 0.100 to 0.085, and extended the range of validity of Amontons' law from 16.5 kg wt to respectively 34, 32 and 26 kg wt (Fig. 1).

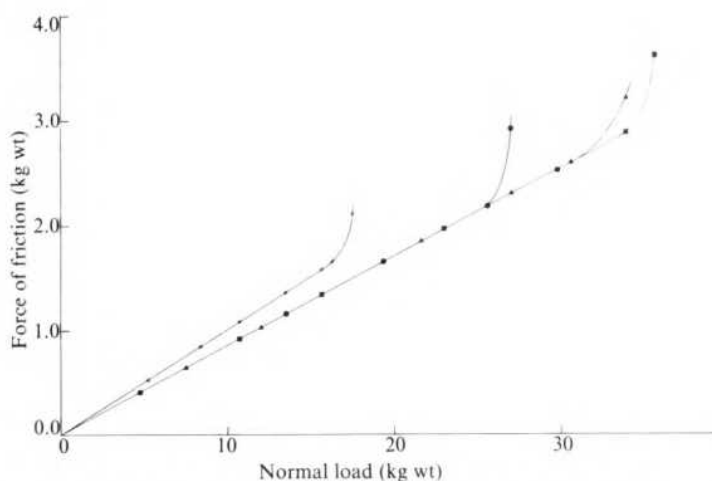


Fig. 1 Force of friction-normal load diagrams (0.9255 cm diameter En-31 steel balls; 1,500 r.p.m.; 30 s runs; additive solutions of optimum concentrations). $\times$, Light mineral oil; $\square$, 5% by wt chlorinated paraffin wax A; $\triangle$, 2% by wt chlorinated paraffin wax B; $\circ$, 1% by wt benzyl chloride (% solutions in light mineral oil).

On this evidence benzyl chloride was less effective than either of the two chlorinated paraffin waxes. The same order of merit was just discernible from the wear scar diameter curves. At 21.6 kg wt for instance, the respective diameters for 30 s runs were 0.330, 0.325, and 0.323 mm. [The bearing pressure of $25,251 \text{ kg wt cm}^{-2}$ at the end of each of these runs was still well within the extreme-pressure range whereas McCarroll and his colleagues had worked so far outside the range of validity of Amontons' law that macroscopic damage had been done and their pressures had fallen to $4,296 \text{ kg wt cm}^{-2}$ and less. Their rate of wear had been nearly 400 times larger than ours.] The corresponding volumes of dust abraded were 1.21×10^{-7} , 1.14×10^{-7} , and $1.11 \times 10^{-7} \text{ cm}^3$. By electron beam induced X-ray analysis considerably

less chlorine was found on wear prints obtained with 1% of benzyl chloride than with the optimum concentration of one of the chlorinated paraffin waxes (Table 1). Wear prints obtained with the base oil alone in the transition region from 'mild' to 'catastrophic wear' (Fig. 2a) resembled those found earlier by Czichos and Kirschke⁴. They had the appearance of a paste having been smeared over the wear print. By contrast, scanning electron micrographs from runs with chlorinated paraffin wax (Fig. 2b) and benzyl chloride (Fig. 2c) in conditions of mild wear showed clearly defined details of the wear scar areas. A striking difference between them was some wear debris sticking to the wear prints obtained with chlorinated paraffin wax (Fig. 2b). These blobs of debris contained more chlorine than the areas around them (Table 1).

Fig. 2 a, SEM photomicrograph ($\times 28,000$) of a wear print on an En-31 steel ball after a 30 s run with a light mineral oil at 15.7 kg wt and 1,500 r.p.m. b, A wear print on an En-31 steel ball after a 30 s run with the optimum concentration (2% by wt) of chlorinated paraffin wax at 21.6 kg wt and 1,500 r.p.m. Blobs of wear debris can be seen on the wear scar. ($\times 2,500$). c, A wear print on an En-31 steel ball after a 30 s run with the optimum concentration (1% by wt) of benzyl chloride at 21.6 kg wt and 1,500 r.p.m. ($\times 2,700$).

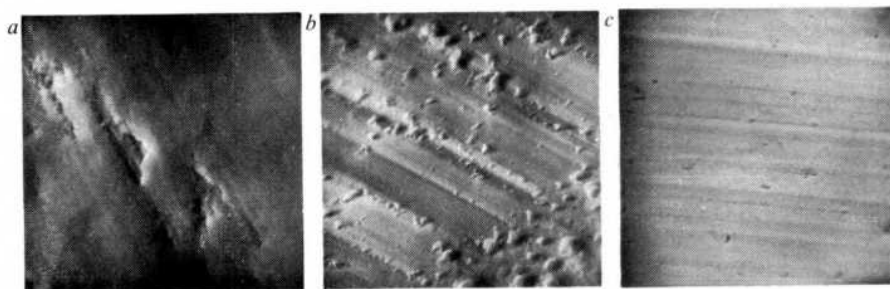


Table 1 Chlorine content found by electron beam induced X-ray analysis of the bases that the contents of Mn, Cr, Fe, and Cl add up to 99.99%

Extreme-pressure additive	Percentage of Cl		
	On wear print area	On blobs of debris	On arbitrarily selected spots
5% by wt chlorinated paraffin wax B	0.38	2.48	—
2% by wt chlorinated paraffin wax B (optimum concentration)	0.20	1.34	—
1% by wt benzyl chloride (optimum concentration)	Trace	—	0.10

Wear prints were obtained from 30 s runs with 0.9525-cm diameter En-31 steel balls at 1,500 r.p.m. and 21.6 kg wt.

We conclude from these observations that chlorinated paraffin wax is superior to benzyl chloride as an extreme-pressure additive. While the chemical reactivity of the additive plays some part in the mechanism of extreme-pressure lubrication, it does not lead to the formation of a wear-protective layer.

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SILVER AND MOULD REPLY—We were rather surprised that Schnurmann and Porter found "quite unacceptable" our view¹ that extreme pressure lubricants containing either sulphur or chlorine form with steel surface layers of iron sulphide or chloride which reduce metal wear and prevent seizure. This is not only our view but also that given by Rowe², Prutton³, Davey⁴ and many other workers in the field of extreme pressure lubrication. We were similarly surprised to read the statement that the chemical reactivity of the additive, although playing some part in the mechanism of extreme pressure lubrication, does not lead to the formation of a wear-protective layer. This too is in contrast with our findings³, and the findings of others^{6,7}, of the relationship between chemical activity and extreme pressure performance.

However, it is possible that a misunderstanding has arisen over the definition of the lubrication condition known as 'extreme pressure'. By this we, and others, mean the conditions whereby lubrication is maintained by means of a surface layer of principally inorganic material. These

conditions are operable under the severe loadings found in hypoid gears or in certain metal cutting operations, and are in contrast with those used by Schnurmann and Porter in their experiments. In lower loaded anti-wear tests⁸, we found that chlorinated hydrocarbons gave a slightly better performance than benzyl chloride, although at the same chlorine concentration in each oil; however, we must question the validity of results obtained from blends of chlorine compounds as described by Schnurmann and Porter, each with such different chlorine contents.

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Interspecific competition in tits

DHONDT has recently made a case for the existence of interspecific competition between blue and great tits¹. My first criticism is mainly statistical and concerns Dhondt's Fig. 3 in which he presents a Lotka-Volterra graph with the equilibrium lines for the two species, estimated subjectively by eight colleagues. I tried a discriminate function analysis as a more rigorous approach and found that the blue tit line had a slight, though insignificant, positive slope. Moreover, in a linear multiple regression of population change of one species on the density of both species, neither species had a significant effect on the other. Consequently, neither the competition coefficients nor the conclusions about niche breadth drawn from the coefficients can be upheld.

My second criticism concerns the interpretation of the significant regressions in Dhondt's Figs 1 and 2. Both figures show that relative to great tits, the reproductive success of blue tits increases as the combined density of the two species increases. Because of the greater variance of the great tit density the result, especially in Fig. 2, could be due to intraspecific, density-dependent effects. In a linear multiple regression of relative competitive ability (great tit fledged per pair divided by blue tit fledged per pair) on year, blue tit density, great tit density, and the ratio of

great tit density to blue tit density, no one independent variable is significant although the whole regression is significant. Thus, the dependence of relative competitive ability on combined density seems uncertain and the possible contribution of the blue tit–great tit ratio should not be rejected. Finally, as there will be interspecific differential mortality, emigration, and so on, there is no reason to think that the equilibrium combined density should be where the reproductive abilities of the two species are equal.

British data support the trends reported by Dhondt except that in contrast to Dhondt's study, there is a strong positive correlation between changes in blue and great tit densities².

I thank M. G. Bulmer, J. R. Krebs, and C. M. Perrins for useful discussions.

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- Dhondt, A. *Nature* **268**, 521–523 (1977)
- Minot, E. O. (in preparation)

DHONDT REPLIES—In my letter¹ I did two things (1) demonstrate that competition for food in the breeding season between great and blue tit results in a reduced fitness (fewer young produced) and (2) speculate how these titmice nevertheless coexist. Minot confirms point (1) but here criticises point (2).

The first part of Minot's criticism concerns the isoclines I tried to estimate. The method I used was unorthodox. I thought that fitting the lines by eye made it possible to correct for aberrant points caused by varying environmental factors. The more orthodox approach is to calculate a multiple regression equation in which the relative change in numbers (not the absolute value as Minot has done) is expressed as a function of each species' density. Doing this, leaving the 1962 point out (see ref. 1), results in isoclines very similar to the ones drawn in my Fig. 3. These are, for the great tit (GT) $1.817 - 0.075 \text{ GT} - 0.045 \text{ BT} = 0$ and for the blue tit (BT) $1.186 - 0.098 \text{ BT} - 0.0087 \text{ GT} = 0$. It is correct that change in numbers of either species is not significantly correlated with the density of the other species. However both partial correlation coefficients are negative and the one expressing the effect of the BT on GT change in numbers is almost significant ($r = -0.42$, $t_{14} = 1.736$, $P = 0.05$, one tailed), although the other one is much lower ($r = -0.23$). We know however, that the key factor, contributing most of the variance to annual mortality is 'overwinter mortality'^{2,3}. Therefore it would be surprising if change in numbers could be related directly to breeding density in the previous year, without correcting for the factors that cause this variance. I conclude

therefore that the competition coefficients should be treated cautiously but need not be rejected. The conclusions about niche breadth were not, as Minot states, derived from the competition coefficients, but inferred from the results in my Table 1, and only refer to the breeding season. I did not imply that, at other times of the year, this situation holds.

In the second part of his criticism Minot concludes that, because BT breeding numbers vary less than GT numbers, there could be a contribution of the relative proportion of each species to relative competitive ability. This may be so at high combined densities, but using only the data where the variance of the two species is not different (my low combined density years, upper line in Fig. 1) my conclusion is not affected.

I am grateful to Minot for pointing out the difficulty of this type of a posteriori data analysis. I think that critical field experiments designed to test hypotheses will contribute more to our understanding of this problem than further manipulation of the data. The first results from such experiments^{4,5} show that outside the breeding season great and blue tit also compete strongly, but that the situation is reversed and that the blue tit is more affected by it than the great tit.

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Cordilleran Benioff Zones

CONEY AND REYNOLDS¹ have interpreted the radiometrically dated igneous rock pattern in the southwestern boundary of North America in terms of the evolution of a convergent plate margin. They conclude that the pattern reflects a progressive decreasing of the Benioff Zone dip angle from about 130–110 Myr to about 55–40 Myr ago and a rapid increasing of dip from about 40 to 15 Myr. Because of these changes in dip, the magmatic arc zone was displaced about 1,000 km inboard from the convergent margin until 55–40 Myr, then swept it back towards the trench by about 20 Myr. The width of this magmatic zone also changes as a function of the dip angle, from narrow at steep angles to very wide at low angles. Although the data seem to be consistent in supporting their interpretation, another interpretation based on the application of the same plate tectonic scheme^{2–5} used by Coney and Reynolds can be proposed.

According to the model of Dickinson and Hatherton^{2–5}, the surface arc mag-

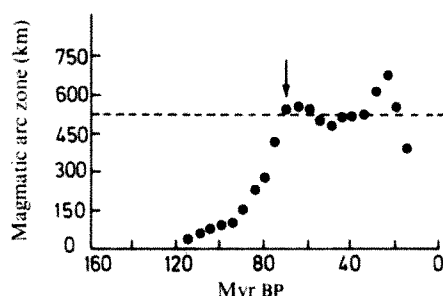


Fig. 1 Growth of the magmatic arc zone. The time when the descending plate approximately reached the 300 km limit of the low-velocity layer is indicated by the arrow.

matism occurs above the zone in which the descending plate intersects the low-velocity layer of the upper mantle. This zone extends from a depth of about 100–300 km. By extrapolating this concept to the earlier stages of development of a magmatic arc, it can be expected that the width of the surface manifestation zone increases as the descending plate reaches greater depths within the low-velocity layer. When the plate reaches the 300 km-limit, if no changes in dip occur, the width of the magmatic arc zone remains nearly constant, until the subduction process ends and the geometry changes. In respect of the zone between 0 and 100 km depth, called the zone of shallow earthquakes, the dip can be different compared to the dip in the low-velocity zone as a consequence of the differences in properties and factors which control the plate interactions in the margin. Thus we can expect two dip angle zones⁶, which have been recognised in several parts^{2–6} and different dip changes in time. At the earlier stages the dip is steep and decreases as the margin evolves until the subduction process finishes or the geometry changes.

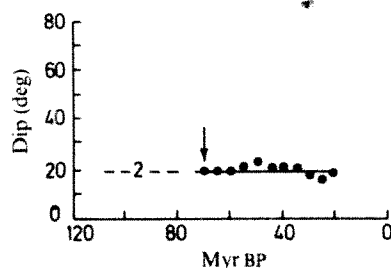


Fig. 2 Magmatic production zone dip angle as a function of time beneath southwestern North America.

By examining the data of Coney and Reynolds, we find that the width of the magmatic zone increases from about 130 to about 70 Myr and remains nearly

constant between 70 and 20 Myr with certain changes from 30 to 20 Myr (Fig. 1). By using a limits of 100 to 300 km for the low-velocity layer and the width of the magmatic arc zone, we can calculate the magmatic production zone dip angle, which is nearly constant from 70 to 20 Myr (Fig. 2). In respect of the zone of shallow earthquakes, the dip angle in this zone experiences a progressive flattening from 130 to about 70–60 Myr, remains constant between 70–60 and 30 Myr, followed by a rapid steepening from 30 to 15 Myr (Fig. 3).

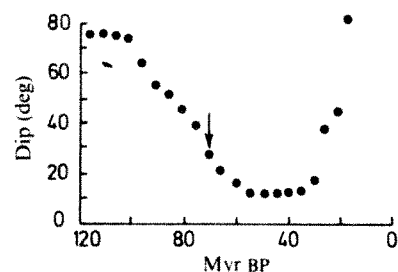


Fig. 3 Shallow earthquakes zone dip angle as a function of time beneath southwestern North America.

The subduction rate during the earlier stages of development can be easily estimated from Fig. 1. The rate increases from 130 to 70 Myr. The changes in the interval between 30 and 20 Myr could represent the evolution of the Kula-Farallon triple junction as suggested in the reconstruction of Atwater⁷. Thus, these changes can be explained as the result of cessation of subduction caused by the change in plates geometry.

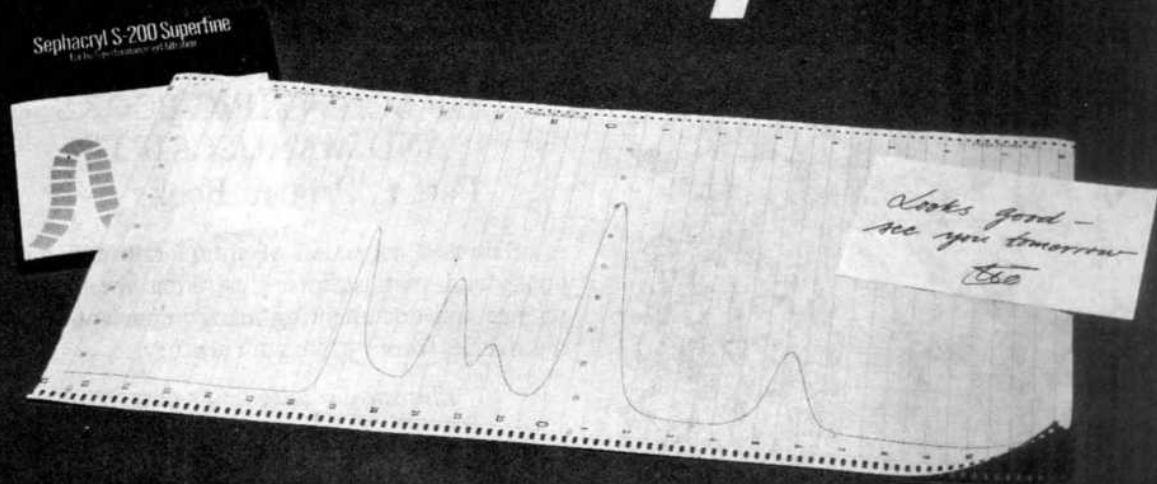
It is expected that the model sketched here represents a more reliable approach to understanding the evolution of the southwestern margin of North America. The qualitative aspects of this model were already included in a work under preparation which includes studies of the metallogeny zoning in northwestern Mexico and the evolution of Baja California peninsula. Further details of the present discussion will also be reported elsewhere.

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reviews

Our energy alternatives

Walt Patterson

Earth, Water, Wind and Sun: Our Energy Alternatives. By D. S. Halacy Jr. Pp. 186. (Harper and Row, New York and London, 1978) £4.95.

Too many commentaries on 'alternative energy' seem to assume that we have only just discovered the sun. What is needed is a sense of historical perspective. It is otherwise too easy to overlook the fact that many ingenious technologies to exploit ambient energy flows like wind and sunlight were developed decades, indeed centuries ago. The principles and even the engineering of such technologies are often better understood than those of so-called 'conventional' energy technologies like offshore oil extraction or nuclear power. This historical perspective is one of several distinctive virtues displayed in D. S. Halacy's book, an outstanding example of an under-rated discipline and popular science writing at its best.

Even for a battle-weary reader case-hardened by continual exposure to outpourings about 'alternative energy', Halacy's book has an exhilarating freshness, and is quite unexpectedly absorbing, partly because of the frequently unfamiliar and fascinating detail included and partly because of the writing itself. The book sustains the reader's interest without gimmicks or gee-whizzery, succumbing neither to the breathless flashiness prevalent in the 1950s nor to the more recently fashionable monitory breast-beating and finger-pointing. Furthermore, Halacy reveals himself to be a virtuoso of the short sentence. His prose races along, delivering an astonishing quantity of information accurately and effortlessly, without a hint of condescension.

He sets the stage with an introductory chapter entitled "The Energy Crisis Is Real", or, as he puts it, "But first, the bad news." It would doubtless be possible to quarrel with the picture he paints, at least in some particulars. He is preoccupied almost entirely with energy supply, and discounts far too completely the opportunities for improved efficiency—for optimising the balance between ambient energy, fuel energy, energy conversion systems, and desired end-use objectives. However, he then moves on to devote

chapters to geothermal energy, water-power, tidal power, sea thermal energy, wind, biofuels, and solar energy; and in each case his survey is both crisply concise and impressively comprehensive. He discusses basic principles, pioneering projects, current plans, proposals and prospects for the future, salting the discussion with striking examples and vivid turns of phrase. You probably know about Palmer Putnam's huge windmill on Grandpa's Knob; but did you know about Georges Claude's sea thermal plant with the intake pipe one and one quarter miles long, off the Cuban coast? In 1927? Or consider this thought-provoking aside, on the theoretical limit to recovery of energy from the wind: "(Extracting all the energy would mean bringing all the wind to a dead stop, an interesting possibility.)".

Halacy also avoids a pitfall into which many of his compatriots topple.

Unlike them he does not seem to believe that energy issues stop at the three-mile limit around the US. On the contrary he draws his examples and his ideas from every corner of the globe, although activities in the US do tend to predominate.

The book also offers a wealth of pictorial accompaniment to the text, including some photos of considerable rarity value.

All told, in 186 pages Halacy has put together a book which can be recommended with enthusiasm as one of the best available introductions to "our energy alternatives". Even its upbeat final sentence is welcome: "It's our move—and the prospects are encouraging." The next time some official spokesman threatens you with freezing in the dark, introduce him to Halacy.

Walt Patterson is energy consultant to Friends of the Earth (London) Ltd.

Ecology of freshwater macrophytes

River Plants: The Macrophytic Vegetation of Watercourses. By S. M. Haslam. Pp. 396. (Cambridge University Press: Cambridge and London, 1978) £27.50.

AGNES ARBER published her outstanding book on water plants nearly 60 years ago. Apart from Fritz Gessner's *Hydrobotanik*, Dr Haslam's is the first dealing with these plants, and based on original research, to have appeared since then. A causal analysis of the distribution of river plants throughout Britain is attempted in terms of flow, substrate and nutrients. These findings are applied to downstream changes in habitat and species composition in whole rivers. This leads to a classification of the vegetation of streams on soft rocks, streams on hard rocks and, finally, of channels with little flow. A preliminary account follows of the vegetation of watercourses in eastern North America and the book concludes with five chapters on applied aspects—uses and hazards created by river plants, their management and their relationship to pollution.

The author clearly intends to interest and indeed educate non-botanists and particularly those engaged in water

planning and management; there is an initial set of drawings of 'field' plants, and symbols are used in text, tables and river maps for the commonest species. Perhaps this explains the way degrees of correlation between nutrient and species distribution are presented (Tables 8.2, 8.6) and the grouping of all moss data as 'mosses'. The professional biologist or teacher may find it difficult to extract succinct information because of inevitable compression of data and, sometimes, insufficient distinction between generalisations based on these data and on subjective assessment. The book is very well produced, with good illustrations, pleasant lay-out and few errors—the most serious being the omission of all data from Fig. 6.12. These minor faults only slightly mar an outstanding, single-handed contribution to river biology—the first classification in terms of their macrophytes of British (and some North American) watercourses. The book can confidently be recommended to all those interested in the ecology of freshwaters.

D. H. N. Spence

D. H. N. Spence is Professor and Head of the Department of Botany at the University of St Andrews, UK.

Origins of senescence

The Genetics of Aging. Edited by E. L. Schneider. Pp.424. (Plenum: New York and London, 1978.) \$39.

DESPITE a considerable amount of research in recent years, as one of the contributors to this volume states, "... a universally acceptable definition of normal biological aging is still not available. The origins of senescence remain enigmatic, and little agreement exists regarding its true nature". Having read this well referenced up-to-date review of the subject one cannot help but agree with this sentiment, but as the nature and cause of aging raise basic fundamental questions in biology perhaps this is not too surprising.

The book is divided into four parts. The first is concerned with the possible molecular basis of aging which resolves essentially into error and programmed theories. The former assumes that aging results from the accumulation of errors in DNA replication or deficiencies in the repair of such errors. The latter assumes that aging results from genetically programmed senescence. Evidence is presented both for and against these two possibilities. The observation in man of increasing hypodiploidy in cultured lymphocytes with age, most frequently affecting the X chromosome in females and the Y chromosome in males, is intriguing, but whether this is a cause or effect of aging remains unresolved.

The second part of the book is concerned with aging at the level of the organism itself from protozoa and fungi to vertebrates. Whether certain findings in lower organisms are of general relevance is a mute point. For example, in *Podospora anserina* (a filamentous fungus) there is clear evidence that longevity is at least partly controlled by cytoplasmic factors. In higher forms life span and survivorship can be expressed in terms of allometric and Gompertz equations for which Sacher presents convincing explanations. It would seem reasonable, however, that aging in man is at least partly determined by genetic factors and the third and largest part of the book is devoted to this subject. In 1961 Hayflick first demonstrated that human diploid somatic cells have a limited replicative life span in tissue culture which is inversely related to the age of the donor. Since then, this has become an exciting and popular experimental tool for studying the phenomenon of aging at least as this is expressed *in vitro*. Detailed consideration is given to this phenomenon in the case of those genetic syndromes known

to be associated with premature aging such as progeria in which fibroblasts in culture show reduced survival, defective HLA expression, increased heat lability of cytoplasmic enzymes, changes in insulin receptors and tissue factors promoting clotting. These observations are appealing; but are such disorders a legitimate paradigm of normal aging? The possible role of genetic factors in aging is more directly approached by family and twin studies. The findings in the now famous Pearl (Baltimore) study of longevity among relatives of nonagenarians are discussed in detail and indicate that there is a definite familial component in aging. However, this is small and Murphy is careful to avoid being dogmatic as to its nature. It could well be largely cultural or environmental. On the other hand, the discussion of

twin studies gives more emphasis to a genetic component in aging.

The fourth and final part of the book is concerned with certain experimental approaches to aging, including somatic cell genetics, immunogenetics and behavioural genetics.

Inevitably in a work of this nature there is some repetition but this is less than in most large multi-authored books. In any event it is often interesting to compare the views of different contributors on the same subject; though the index itself is often unhelpful in this regard. This is a highly readable and well referenced review of a fascinating subject and I would recommend it without reservation.

Alan E. H. Emery

Alan E. H. Emery is Professor of Human Genetics at the University of Edinburgh, UK.

Nuclear transplantation experiments in Amphibia

Cloning: Nuclear Transplantation in Amphibia. By R. G. McKinnell. (University of Minnesota Press: Minneapolis, 1978.) \$22.50.

IN spite of its principal title, this book is not concerned with most aspects of cloning. Thus, it does not deal with cloning in plants, with the cloning of DNA or genes in bacteria by plasmids and phages, nor with the cloning of animal cells in culture. In fact the book consists entirely of an account of nuclear transplantation experiments in Amphibia, primarily those with *Rana pipiens*. This has its use, and a chapter on the transplantation of nuclei from tumour cells is clearly presented and well balanced.

Unfortunately the same cannot be said for much of the rest of the book. In general, work with species other than *R. pipiens* is treated as trivial or at best confirmatory. Often it is hard to discern the direction of an argument. Thus, the book does not make it clear that many amphibian nuclear transfer experiments have been addressed to the specific question of whether the nucleus of one kind of specialised cell contains the genetic information necessary to promote the formation of other quite unrelated cell types. Though generally considered important, this question is totally obscured in this book by the author's decision to emphasise the age rather than differentiated state of donor cells.

From the student's point of view, a

serious deficiency is that alternative but not exclusive interpretations are often omitted. For example, most of the first half of the book is concerned with abnormal embryos resulting from nuclear transfers in *R. pipiens*. It is therefore disappointing to find that one of the principal ideas regarding the significance of these abnormal embryos is entirely overlooked. The author presses the view that the abnormalities of nuclear transplant embryos may give specific information about the specialised state of gene control in donor cells. An alternative interpretation (favoured by several in this field) is that abnormal embryos result from random chromosomal losses due to the low replication rate of specialised cells and the very high rate at which eggs always divide. My criticism is not that the author subscribes to the former point of view, but to disguise or ignore the second interpretation seems to me to significantly misrepresent the literature.

The last half of the book contains useful technical information in the form of six appendices. These deal with the biology and maintenance of *R. pipiens*, the instruments, solutions and techniques used to transplant single nuclei to eggs (primarily *R. pipiens*), as well as with sources of various anuran species.

The unbalanced presentation and uncritical interpretation of nuclear transplantation experiments seriously limit the general usefulness of the first part of this book. However, the technical information in the appendices should prove valuable for those wishing to transplant nuclei in *R. pipiens*.

J. B. Gurdon

J. B. Gurdon is at the MRC Laboratory of Molecular Biology in Cambridge, UK.

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This book presents the proceedings of the symposium "Developments in Clinical Immunology" organized by Professor Mario Ricci, Director of the Postgraduate School of Allergology and Clinical Immunology and held at the University of Florence. The aim of the conference was to bring together eminent scientists interested in both the theoretical and clinical aspects of immunology. Their papers provide a summary of the state of the art and of the recent advances that have been made in the field of atopy and lymphocytes. Undergraduates and graduate students will find it invaluable to have such a wealth of material gathered together under one cover sparing them from searching through diverse journals of allergy, immunology, biochemistry, physiology and pathology where such information is usually disseminated.

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Football fans, Maori children, factory shop stewards or engineering students — in spite of their apparent incongruities as common subjects for a single study — each possess a group identity and display complex intergroup relations, the description of which is one of the aims of this book, about the new theory and research related to it. How do social mobility, change or rigidity, conformity or deviance, self-identity versus group categorization, and majority or minority status affect inherent tendencies for people to differentiate themselves one from another? The basic thesis of this book is that there is a marked tendency to social differentiation rather than conformity, and that this tendency, as a national, ethnic, linguistic or individual level, both promotes social innovation and creativity and, equally, becomes a source of conflict and waste.

Metal β -Diketonates and Allied Derivatives

R. C. Mehrotra, R. Bohra and D. P. Gaur

October/November 1978, viii + 832pp., £20.50/\$42.40 0.12.488150.5

Presented here is a concise account of the developments made in different eras in the chemistry of metal β -diketonates. The book concentrates on four aspects: their synthesis, the nature of bonding and their aromatic character, their chemical reactions and their physical properties, including spectroscopic, magnetic and structural characteristics. There are now more than a hundred research papers published annually on different aspects of this fascinating field of chemistry. The present monograph covers around two thousand references to original literature and the authors have attempted to include all the major breakthroughs and developments of this fast growing subject.

Mammalian Metabolism of Plant Xenobiotics

R. R. Scheline

October/November 1978, xli + 502pp., £28.00/\$58.00 0.12.623350.0

Plant xenobiotics, which can roughly be equated with those compounds termed 'secondary plant metabolites', are of considerable importance in many fields of research such as pharmacology, toxicology, medical and natural products chemistry, and food sciences. It is important therefore that information on their metabolites and the pathways leading to them, is readily available to those working in such active areas of research. A large quantity of literature on xenobio-chemistry has been published in recent years, but references are scattered and location of an overall summary of the metabolism of a specific compound is often difficult and time consuming. Accordingly, this volume has been compiled to provide a comprehensive survey of the current knowledge in a specific part of this large field, namely that of plant xenobiotics.

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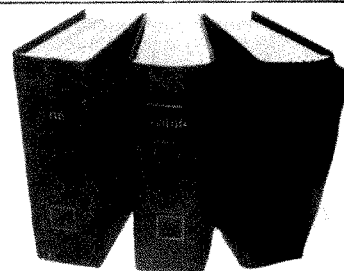
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APPOINTMENTS VACANT

THE UNIVERSITY OF LEEDS DEPARTMENT OF PLANT SCIENCES

Applications are invited for the temporary fixed-term post of post-doctoral RESEARCH FELLOW to work with Dr L. D. Incoll on a project concerned with work on when and how cytokinins open graminaceous stomata.

Ideally applicants should have been trained at the postgraduate level in research on phytohormones and should have research experience in that field. Appointment is for one year in the first instance, renewable for up to a total of three years. Initial salary will be in the range £3,883 to £4,382 per annum (within the 1A range of the scale for Research and Analogous staff). (Under review.).

Applications, giving curriculum vitae and the names of two referees, should be sent by October 5, 1978 to Dr L. D. Incoll, Department of Plant Sciences (Baines Wing), The University of Leeds, Leeds LS2 9JT, from whom further details may be obtained. Closing date for applications has been revised to October 20, 1978, in view of the fact that this advertisement did not appear in the journal at the appropriate time. 7073(A)

THE UNIVERSITY OF LEEDS DEPARTMENT OF METALLURGY

Applications are invited for the post of POSTDOCTORAL RESEARCH FELLOW in Photo-Emission Electron Microscopy to participate in a wide range of physical metallurgical work in the Department, with special emphasis on high temperature and deformation phenomena. The successful applicant will be responsible for the organisation of work carried out on the Photo-Emission Electron Microscope together with the training of associated technical staff and research students who will operate it. Candidates should have a Ph.D. in a relevant scientific or engineering field or be in the process of completing their thesis. Previous research experience involving electron microscopy and/or the use of vacuum equipment would be an advantage.

Initial appointment will be made at an appropriate point in the range £3,883 to £4,631 (under review) on the 1A Scale for Research and Analogous Staff.

Applications, with the names of two referees, should be sent to Professor J. Nutting, Department of Metallurgy, The University of Leeds, Leeds LS2 9JT by October 16, 1978. 7307(A)

HERIOT-WATT UNIVERSITY RICCARTON

MARINE SCIENCE UNIT
INSTITUTE OF OFFSHORE ENGINEERING
RESEARCH ASSOCIATES (THREE POSTS)
Salary scale £3,384 to £4,882 (under review)

RESEARCH TECHNICIAN
Salary in range £2,688 to £3,720 (under review)

Applications are invited for the above posts. The successful applicants would join a team investigating the nature, fate and effects of hydrocarbons and other pollutants in industrial effluents, shellfish and marine sediments, under programmes supported by industrial contract and research grants.

Applicants for the Research Associate posts should have an honours degree in chemistry or biochemistry, preferably with experience in chromatographic (notably gc) techniques. Driving licence and/or ability to dive (SCUBA) desirable but not essential.

Applicants for the post of Research Technician should have appropriate educational qualifications in chemistry and experience in handling and maintenance of high resolution chromatographic equipment.

Informal enquiries may be made to Dr C. S. Johnston, Director of the Marine Science Unit, telephone 031 449-5111, Ext. 2382.

Application forms are available from the Staff Office, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX, to whom the completed forms should be returned as soon as possible. 7323(A)

Microbiologist £2,839 - £6,898

Applications are invited for a pensionable post in the Department of Agriculture's Agricultural and Food Bacteriology Division, Newforge Lane, Belfast.

The successful candidate will be expected to undertake research and investigational work connected with the microbiology of foods and may also be required to undertake lecturing duties in the Faculty of Agriculture and Food Science, Queen's University, Belfast.

Appointment may be at Senior Scientific Officer, Higher Scientific Officer, Scientific Officer level.

SENIOR SCIENTIFIC OFFICER

Over 25 and under 32 years of age on 31 December 1978 with a 1st or 2nd class Honours Degree in Microbiology, and at least 4 years appropriate postgraduate experience.

HIGHER SCIENTIFIC OFFICER

Under 30 years of age on 31 December 1978 with an Honours Degree as above and at least 2 years appropriate postgraduate experience.

SCIENTIFIC OFFICER

Under 27 years of age on 31 December 1978 with an Honours Degree as above.

Exceptionally applications may be considered from candidates over the age limits who have specialised experience.

The Civil Service Commissioners may decide to interview only those applicants who appear from the information available (including level of academic attainment) to be best qualified.

SALARY SCALES

Senior Scientific Officer — £5,154-£6,898

Higher Scientific Officer — £4,101-£5,448

Scientific Officer — £2,839-£4,415

Grading and starting salary will be related to qualifications and experience.

Please write or telephone for an application form quoting job reference SB 359/78/N to the Civil Service Commission, Rosepark House, Upper Newtownards Road, Belfast BT4 3NR (telephone Dundonald 4585 Ext 257). Completed forms must be returned to arrive not later than 19th October 1978. 7345(A)



M.R.C. CLINICAL RESEARCH CENTRE (Northwick Park Hospital)

Watford Road, Harrow, Middx. HA1 3UJ

Technician required to operate and maintain a gas chromatography-mass spectrometry-computer facility in the Division of Clinical Chemistry.

Applicants should have a primary interest in instrumentation and electronics and preferably have a general background in analytical chemistry. Previous mass spectrometry experience would be an advantage but is not essential. Minimum academic standard required would be an HNC in physics, chemistry or related subject.

Salary within the range £3,615 to £5,034 incl. L.W. Application forms and job description from Mrs J. Tucker-Bull quoting reference 106/2/4004. Closing date October 20th.

7338(A)

THE UNIVERSITY OF BRITISH COLUMBIA

Department of Chemistry

POSTDOCTORAL RESEARCH POSITIONS

Applications are invited from well qualified candidates for one or two year appointments as Postdoctoral Research Fellows in the following fields:—

Atmospheric photochemistry; Bioinorganic chemistry; Catalysis studies, diffusion and phase separation on surfaces; Catalysis using transition metal complexes; Chemical Oceanography; Development of New Synthetic Methods in Organic Chemistry; Electron impact and Photoelectron Spectroscopy including Auger and ESCA studies; Electron paramagnetic, ENDOR, NQR, Mossbauer, microwave, electronic and LEED spectroscopies; Flash photolysis and Kinetic Spectroscopy; Fluorocarbon Chemistry; Homogeneous Catalysis; Immunochemistry of polysaccharides; Inorganic photochemistry and photo-physics; Kinetic theory of gases applied to aeronomy and space science; Lipid synthesis for NMR study of biomembranes; Mechanism of Intercalation of layered Structures; Muonium Chemistry at TRIUMF; NMR and laser Raman spectroscopy of nuclear spin labelled nucleic acids; Non-linear optical effects in organic solids at low temperature; Nuclear magnetic resonance studies of molecular motion; Organic Solid State Reactions; Organometallic Chemistry; Oxidation, use of coenzyme models, general acid catalysis; Potential antiviral and antitumor compound synthesis; Quantum chemistry and Molecular Physics; Radiation chemistry and solvated electron studies; Synthesis and study of organic metals; Theory of molecular relaxation and transport properties; X-ray diffraction studies.

The minimum stipend for the first year will be \$11,100. A substantial number of Teaching Postdoctoral Fellowships, with a minimum stipend of \$13,750, are also available. Killam Postdoctoral Fellowships, with stipends of \$14,000, are open to students who have recently graduated and have shown superior ability on research and scholarship.

Send curriculum vitae, transcripts, and letters from three referees to the undersigned, from whom further particulars may be obtained.

Professor C. A. McDowell
Department of Chemistry
**UNIVERSITY OF
BRITISH COLUMBIA**
Vancouver, B.C. V6T 1W5
CANADA

7311(A)

AGRICULTURAL RESEARCH COUNCIL INSTITUTE OF ANIMAL PHYSIOLOGY BABRAHAM, CAMBRIDGE CB2 4AT

RESEARCH SCIENTIST

required in the Physiology Department to carry out research into biologically-active peptides and proteins (e.g. gastro-intestinal hormones, somatomedins, hypothalamic peptides), their isolation, characterisation and mechanism of action. Salary in H.S.O. (£4,101 to £5,448 per annum) or S.S.O. (£5,154 to £6,898 per annum) scale according to experience. Candidates should have first or upper second class honours degree in physiology or biochemistry, with at least two years' relevant postqualifying experience. Preference for Ph.D. Non-contributory superannuation scheme. Applications to the Secretary of the Institute by October 30, 1978. Quote Ref. A.M.B.3. 7299(A)

THE POLYTECHNIC OF CENTRAL LONDON Bio-organic Research Group RESEARCH ASSISTANT IN MICROBIAL ENZYMOLOGY £3,015 to £3,189

To work on the cellulose-degrading enzymes produced by bacteria. The successful candidate will join a small team of biotechnologists working on the production and properties of cellulases.

Applicants should have a good class degree in an appropriate subject and will be able to register for a higher degree.

Further details and application form, which should be returned as soon as possible, from the Establishment Officer, P.C.L., 309 Regent Street, London W1R 8AL (tel. 01-580 2020, ext. 212). 7300(A)

INSTITUTE OF DERMATOLOGY (University of London)

require a

TECHNICIAN

to work in the laboratories of the Professorial Unit. The successful candidate will work on the development and use of radioimmunoassays for pharmacological mediators related to inflammation in human skin. Preference will be given to applicants with experience in radioimmunoassay techniques.

Applications, giving details of age, qualifications, experience and the names of two referees should be sent to Dr N. A. Plummer, Institute of Dermatology, Homerton Grove, London E9 6BX. 7302(A)

ROYAL MARSDEN HOSPITAL FULHAM ROAD, LONDON SW3. Medical Laboratory Scientific Officer—Immunology £3,615 to £5,034

Applications are invited from medical laboratory scientific officers experienced in tissue typing, for a post which is available immediately. The duties include screening and identifying HLA antibodies, typing of patients and potential donors for the bone marrow transplantation programme, and participation in regional and international workshop exchanges of antisera and information. New developments include the identification of B-cell antibodies and the application of these to studies in malignant disease.

Application forms from Personnel Dept. Tel. 352 8171, ext. 447. Closing date: October 16, 1978. 7295(A)

THE CHINESE UNIVERSITY OF HONG KONG FACULTY OF MEDICINE

Applications are invited for the following foundation chairs:

ANATOMY PHYSIOLOGY PHARMACOLOGY

tenable at this new medical school with an annual student intake of 100. There will be opportunities for original research but considerable undergraduate teaching experience is essential. Non-medically qualified candidates may apply but preference will be given to those medically qualified. Successful applicants are expected to be in post latest end of 1980 or early 1981, to take part in compiling the basic medical sciences curriculum and installing departmental facilities. Teaching will begin in the fall term of 1981. The minimum salary for Professorships will be HK\$11,735 per month (£1=HK\$9.2). Terms of service also include long leave, superannuation benefits (University 15%, appointee 5%), housing accommodation on campus and air passages. Full particulars of the appointment, together with copies of relevant documents concerning the medical school and the University may be obtained from the Secretariat, The Chinese University of Hong Kong Shatin, New Territories, Hong Kong, to which applications (6 copies) giving full details of qualifications and experience and the names and addresses of 3 persons to whom reference may be made should be sent not later than December 15, 1978. 7251(A)

Job Announcement

ARMAUER HANSEN RESEARCH INSTITUTE (A.H.R.I.) INSTITUTE DIRECTOR (LEPROSY RESEARCH)

The position of Institute Director will be available June 1, 1979 or earlier at the Armauer Hansen Research Institute (AHRI), Addis Ababa, Ethiopia. AHRI undertakes basic research on the etiology, pathogenesis and immunology of leprosy. The Institute was founded in 1969 and is operated by the Save the Children organisations of Norway and Sweden.

The Institute is well-equipped for research within the areas of microbiology and immunology, and is located at the All Africa Leprosy Rehabilitation and Training Centre (ALERT) which makes it possible to do clinically oriented studies on patients in ALERT's hospital. The Institute staff consists at present of three senior researchers, a research assistant, two research fellows, a laboratory engineer, a lab technician, four lab assistants, a chief administrator, two secretaries and other personnel—altogether 24 persons.

The Institute Director is responsible for the operation of the Institute as well as leading the research work. Applicants should have well-documented background in microbiological and/or immunological research, preferably a medical degree, although this is not an absolute requirement. Applicants normally must agree to a contract of at least two years.

Further information concerning this position can be obtained from Professor Morten Harboe, Institute for Experimental Research, Ullevål Hospital, Oslo 1, Norway. Telephone 472/60 03 90.

Applications accompanied by a curriculum vitae, a list of publications and personal references should be sent before November 1, or within three weeks after the appearance of this announcement to REDD BARNA (Norwegian Save the Children), Lillestorg 1, Oslo 1, Norway. 7301(A)

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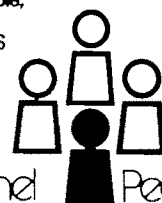
You will be in your 20s or early 30s, educated to at least 'A' level standard and with an appropriate technical background gained from radiography, nursing or as a representative for medical equipment and disposables. You will be expected to become an expert and advisor in the medical applications of the Company's products.

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Your work will also involve you in the sales of the whole range of Polaroid's technical films and systems in a home counties based sales area. The experience you will gain will provide you with the key to the promotion in sales and marketing in this go ahead Company.

The opportunities for any woman or man are limited only by their ability and drive - Polaroid have a vested interest in your success! On offer are first rate salary and car (Cortina 1600GL Estate), progressive benefits, knowledge and relocation assistance if appropriate.

Find out more from David Woolf on Hertford (0992) 50541, or write to him for an application form to The Personnel People, 95 Fore Street, Hertford SG15 1AS.



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7381(A)

UNIVERSITY OF GEORGIA ATHENS, GEORGIA

Applications are invited for the post of

POSTDOCTORAL RESEARCH ASSISTANT

Tenable from October 15, 1978 for one year, for the study of the breakdown metabolites of a plant growth regulator. Applicants should preferably have experience in plant biochemistry and radioisotope technique.

Salary in the range of £6,000 to £6,500 per annum plus University of Georgia faculty benefits.

Applications, including a curriculum vitae and the names of two referees should be addressed to Dr S. J. Kays, Department of Horticulture, Plant Science Building, University of Georgia, Athens, Georgia 30602.

7340(A)

UNIVERSITY OF SOUTHAMPTON FACULTY OF MATHEMATICAL STUDIES CHAIR OF OPERATIONAL RESEARCH

Applications are invited for the Chair of Operational Research. Salary within the professional range. Applicants should have an established reputation in the field of Operational Research. Further particulars may be obtained from the Secretary and Registrar, The University, Southampton SO9 5NH, and applications (11 copies from applicants in the United Kingdom) must be submitted before November 28, 1978. Please quote ref MA2/1/Na.

7331(A)

CENTRO DE INVESTIGACIONES Y DE ESTUDIOS AVANZADOS Mexico City, Mexico

Two positions exist in the Department of Genetics and Molecular Biology for Assistant/Associate Professors working with pro- or eukaryotic microorganisms. Laboratory facilities are excellent; teaching responsibilities minimal; knowledge of Spanish is not necessary. Applicants should possess a Ph.D. Send curriculum vitae, names of three references, synopsis of research and reprints to: Dr Jose Ruiz Herrera, Depto de Genética y Biología Molecular, C.I.E.A. del I.P.N., Apartado Postal 14-740, Mexico 14, D.F.

7281(A)

ROTHAMSTED EXPERIMENTAL STATION Harpenden, Herts, AL5 2JQ ORGANIC CHEMIST

Applications are invited from synthetic organic chemists for a three-year position. The appointee will be expected to synthesise analogues of amino acids and will be involved in their use both at Rothamsted and at the commercial concern sponsoring the project.

Qualifications: 1st or upper 2nd class honours degree in a relevant discipline with at least three years' research experience in organic synthesis. Preference will be given to candidates with a Ph.D. in a relevant subject.

Appointment in grade of Higher Scientific Officer (£4,101 to £5,448) Non-contributory superannuation.

Applications, giving names and addresses of two referees and quoting reference 352 to the Secretary by October 19. Further details on request.

7370(A)

AGRICULTURAL RESEARCH COUNCIL INSTITUTE OF ANIMAL PHYSIOLOGY, BABRAHAM, CAMBRIDGE CB2 4AT BIOCHEMIST

required to join group studying the structure and distribution of antigen determinants in cell membranes and their role in transport processes. Applicants should have an interest in immunology and preferably experience in the preparation and use of conjugated antisera, and/or analysis of glycoproteins. Preference will be given to candidates possessing, or expecting to possess, a Ph.D. or equivalent. The appointment, which is financed by a grant from the M.R.C. is for three years commencing as soon as possible. Salary in scale £4,101 to £5,448 per annum according to qualifications and experience. Applications together with the names of two referees to the Secretary of the Institute from whom further information may be obtained. Ref E.M.T.6

7292(A)

KINGSTON POLYTECHNIC SCHOOL OF GEOLOGY RESEARCH ASSISTANT

to work on the sedimentology and related volcanicity of the middle caradocian rocks in Snowdonia, North Wales. Applicants should have a good honours degree in Geology, including the techniques used in sedimentology and structural geology, and should preferably have some knowledge of volcanic rocks.

The research will be conducted under the supervision of J. N. Diggins and Dr P. J. C. Sutcliffe.

The successful candidate will be expected to register for a higher degree. Salary range £2,910 to £3,084 per annum.

Further details and application forms from Academic Registry, Dept AO, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE 01-549 1366.

7341(A)

QUEEN CHARLOTTE'S MATERNITY HOSPITAL Goldhawk Road, London, W6 BASIC GRADE OR SCIENTIFIC OFFICER (Cytogenetics)

Applications are invited from Science Graduates with honours degrees for the above posts. Preference will be given to candidates with experience of diagnostic work in human cytogenetics. Whitley Council terms and conditions of service. The diagnostic laboratory, which is fully equipped, may be visited by appointment. Intending applicants are advised to contact Dr D. Hughes (Tel: 01-748 4666, Ext: 223) in the first instance.

Application forms obtainable from and returnable to the Administrative Assistant (Ext. 204).

7329(A)

MEDICAL LABORATORY SCIENTIFIC OFFICER (OR JUNIOR)

required for varied endocrine analytical work in DEPARTMENT OF GYNEACOLOGY (Biochemistry Laboratory) H.N.C. standard needed or 'A' level chemistry for junior post. Apply giving age and details of qualifications and experience to Dr M. G. Brush, St Thomas' Hospital Medical School, London SE1 7EH.

7282(A)

Medical Research Council Cell Biophysics Unit

Appointment of Director

The present Director of the MRC Cell Biophysics Unit, Professor M. H. F. Wilkins, CBE, has indicated his wish to resign in the near future. The Council now wish to proceed to the appointment of a Director to succeed him.

The Unit is accommodated in the Department of Biophysics, King's College, London, with which it has a close relationship and shares a number of common facilities. The work of the Unit is to be centred on the biophysical, biochemical and structural study of the mechanisms involved in the control of cell shape and motility (currently with particular reference to the nervous system and to skeletal muscle) but this need not exclude any other aspects of research in cellular biophysics to take into account the Director's own interests. The person appointed as Director, who will be a full-time member of the Council's staff, must have an established reputation as a scientific investigator in a relevant field and the ability to play a leading role in initiating and managing research using a variety of disciplines and techniques. An appointment to the Council's staff will be in accordance with the Council's terms and conditions of service, with superannuation provision under the MRC pension scheme. The salary will be within the range for the Council's Special Appointments grade, which is equivalent to the university professorial range although, exceptionally, a higher salary might be agreed.

Applications for the post are now invited from men and women, and should comprise a *curriculum vitae*, a list of publications, the names of 3 persons to whom reference might be made, and a short statement (not exceeding 500 words) outlining in general terms the scientific programme that the applicant would wish to undertake and indicating how it would relate to the central theme of the Unit's work. These details (12 copies) should be submitted not later than November 10, 1978 to the Secretary, Medical Research Council, 20 Park Crescent, London W1N 4AL. Further information about the appointment and the Unit, including details of its present programme, may be obtained from Dr M. B. Kemp, at the same address (tel: 01-636 5422). The Council reserves the right to invite applications from persons other than those who respond to this advertisement. 7380(A)

MRC
Medical Research Council

THE UNIVERSITY OF BRITISH COLUMBIA DEPARTMENT OF ZOOLOGY

Applications are invited for the position of Assistant Professor in the Department of Zoology, University of British Columbia. Qualifications include a Ph.D. in biology or a related field, with a demonstrated ability to teach introductory biometrics. The successful applicant will be expected to develop a strong experimental research programme. Upper level undergraduate teaching, and graduate student teaching and supervision is required. Salary will be commensurate with experience and duties. Curriculum vitae and the names of three referees should be submitted to Dr G. G. E. Scudder, Head, Department of Zoology, University of British Columbia, 2075 Westbrook Mall, Vancouver, B.C. V6T 1W5, Canada. The expected date of appointment is July 1, 1979, subject to budgetary approval. Closing date for application is when position is filled. 7305(A)

UNIVERSITY OF WARWICK RESEARCH TECHNICIAN

The Department of Biological Sciences has vacancies in the Microbiology Research Group for technicians to work on the isolation and purification of enzymes from methane oxidising bacteria.

Applications are invited from graduates and others with appropriate qualifications and experience in biochemistry and/or microbiology. One of the posts is for a contract period of 2 years.

Salary will be either on the Technician Grade 2 scale: £2,688 to £3,060 p.a. (both under review).

Application should be made by letter giving full details of age, qualifications, experience, etc., to the Personnel Office, University of Warwick, Coventry CV4 7AL by October 13, 1978. Please quote Ref. No: 9/2D/78. 7373(A)

UNIVERSITY OF GLASGOW POSTDOCTORAL RESEARCH ASSISTANTSHIP

Applications are invited for a University Research Assistantship to be filled at postdoctoral level. The post is with a group studying the molecular biology of antibody formation with emphasis on the application of recombinant DNA technology. Experience in molecular immunology or recombinant DNA techniques is desirable.

The position is available from October 1, 1978.

Salary will be within Range 1A of the salary scales for Research and Analogous Staff (£3,883 to £6,555, under review) with appointment at a point appropriate to age and experience.

Application with curriculum vitae and names of two referees should be sent as soon as possible to Professor A. R. Williamson, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ.

In reply please quote Ref. No. 4248M. 7342(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF HISTOLOGY AND CELL BIOLOGY (MEDICAL) SENIOR RESEARCH ASSISTANT

Applications are invited for the above post to join a group studying the interrelationships between the cell surface, the cytoskeleton and the extracellular matrix. Experience or an interest in electron microscopy will be an advantage. The appointment will start as soon as possible and in the first instance will be for one year only.

Initial salary £3,883 or £4,133 per annum.

Applications, together with the names of two referees, should be received not later than October 20, 1978, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref: RV/947/N. 7347(A)

NEWCASTLE UNIVERSITY HOSPITALS ROYAL VICTORIA INFIRMARY NEWCASTLE UPON TYNE POSTDOCTORATE SCIENTIFIC OFFICER

required for three year project concerned with clinical and experimental study of cellular immunology in lymphoproliferative disease. The post is available immediately and will be run in Department of Haematology, in the main Newcastle teaching hospital.

Previous laboratory experience in cellular immunology tissue culture or serology is essential.

Starting salary £4,130.
Contact Dr S. J. Proctor, Department of Haematology, Royal Victoria Infirmary, Newcastle upon Tyne (telephone 25131, extension 7). 7348(A)

KINGSTON POLYTECHNIC SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES RESEARCH ASSISTANT

Applications are invited from candidates with a good honours degree in or including Physics, to study the ultrasonic production of cavitation and its effects on living cells. The successful applicant will be expected to register for a higher degree.

Salary range £2,910 to £3,084 inclusive.

Application forms and further details from Academic Registry, Dept. AO, Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel: 01-549 1366. 7367(A)

Re-advertisement SHEFFIELD CITY POLYTECHNIC DEPARTMENT OF BIOLOGICAL SCIENCES RESEARCH ASSOCIATE IN BIOLOGICAL SCIENCES

Salary Scale: £4,101 to £6,558

This is one of two posts of Research Associate created in the Polytechnic to provide leadership and direction of research in specific areas. The research activities of the Department are concentrated mainly in the areas of Microbial Biochemistry, Animal Physiology and Biochemistry.

Applicants must be well qualified and experienced in one of the above areas and have previous experience of research supervision. Recent industrial experience would be advantageous.

The appointment is for a fixed period of three years.

Requests for an application form in writing only please to the Recruitment Section of the Personnel Department, Sheffield City Polytechnic, (Dept. NA), Halfords House, Fitzalan Square, Sheffield S1 2BB. Completed forms should be returned by October 20, 1978. 7324(A)

UNIVERSITY OF SOUTHAMPTON LABORATORY TECHNICIAN (Physiology)

Applications are invited for an appointment as a Laboratory Technician (Physiology) in the Neurophysiology Group of the School of Biochemical and Physiological Sciences. The successful applicant will be required to assist in the running of Physiology undergraduate and postgraduate (M.Sc.) practical classes and with maintenance and operation of the relevant laboratories. Duties will include some technical support for research in Neurophysiology and allied disciplines.

Minimum qualifications: O.N.C./O.N.D. or equivalent with three years relevant experience. The appointment will be made at grade three on salary scale £2,688 to £3,060 per annum (under review).

Applications giving date of birth, qualifications and experience and the names and addresses of two referees, should be sent to Mr C. N. Saul, The University, Southampton SO9 5NH quoting reference number 482/T./Na. 7337(A)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY RESEARCH ASSOCIATE

Applications are invited for a post of Research Associate (preferably postdoctoral) to join an M.R.C. Programme Grant Project team investigating urinary enzyme excretion in normal and complicated pregnancies. Candidates should have a higher qualification and previous experience in enzyme biochemistry, preferably in relation to renal metabolism. The post is available for up to five years. Commencing salary within the range £4,130 to £5,506 per annum on the National Range 1A scale. Membership of the appropriate University Superannuation Scheme will be required.

Applications giving full curriculum vitae and the names and addresses of two referees should be submitted to Dr J. M. Davison, Princess Mary Maternity Hospital, Newcastle upon Tyne NE2 3BD within three weeks of the appearance of this advertisement. 7321(A)

Nature Conservancy Council

**SCIENTIFIC/HIGHER
SCIENTIFIC OFFICER****SALARY SCALE**

Scientific Officer - - - £2,839 - £4,415

Higher Scientific Officer - £4,101 - £5,448

Applications are invited for the post of Scientific Officer/Higher Scientific Officer in the Nature Conservancy Council Geological Conservation Review Unit Based at Newbury, Berkshire.

The geological work of the Nature Conservancy Council includes the identification and conservation of those localities that are essential to the continued study of geology and physiography; the provision of advice and dissemination of knowledge, both within and outside the NCC; and liaison with interested bodies and individuals. The duties do not include geological and geomorphological research.

THE POST

The Unit is responsible for the preparation of the Geological Conservation Review. The successful candidate will act as Assistant Head of the Unit, taking special responsibility for the localities of Quaternary interest. He/she will be liable, however to secondment to the G & P Section specifically dealing with case work, advisory duties and liaison related to the conservation of localities of Quaternary interest.

AGE AND QUALIFICATIONS

All candidates should have a first or good second class honours degree, or equivalent in Geology and/or Physical Geography with special emphasis and interest in Quaternary studies. A knowledge of photography is desirable.

SCIENTIFIC OFFICERS: Candidates should be under 27 years of age.

HIGHER SCIENTIFIC OFFICERS: Candidates should be under 30 years of age and have at least two years relevant post-graduate experience.

A current driving licence is essential.

Application forms and further details are available from Recruitment Section, Nature Conservancy Council, Godwin House, George Street, Huntingdon, Cambs, PE18 6BU (Tel: 0480 56191).

Closing date for completed application forms is 3 November 1978.

7280 (A)

Tropical Products Institute, London

Food Technologist

■ Investigate preservation methods of fish and meat in tropical countries ■ Concentrate on utilisation of fish waste and animal by-products of tropical origin ■ Occasional participation in overseas projects.

☐ Degree or equivalent in appropriate discipline ☐ Proven research experience and ability to work independently essential ☐ Overseas experience an advantage ☐ Age normally under 32 ☐ Appointment as Senior Scientific Officer (£5620-£7360) or Higher Scientific Officer (£4560-£5910) according to qualifications and experience ☐ Ref: SA/90/JD.

■ Application forms (for return by 26 October 1978) from Miss C. A. Hall, Tropical Products Institute, 127 Clerkenwell Road, London EC1R 5DB. 7372(A)

**Science
Group**
CIVIL SERVICE

NEW ZEALAND

Ministry of Agriculture and Fisheries

Agricultural Research Division

A vacancy exists in the Agricultural Research Division for the position of Director of the Ruakura Animal Research Station. Ruakura is adjacent to Hamilton, a city of approximately 90,000 located 80 miles south of Auckland.

DUTIES:

Responsible for the operation and administration of the Ruakura Animal Research Station. The Station studies general principles of animal production and develops practices aimed at increasing the efficiency of conversion of feed stuffs (predominantly grazed pasture) to meat, milk and wool. Attention is given also to diseases such as facial eczema, bloat, ryegrass staggers and other metabolic disorders related to management of ruminants on pasture.

QUALIFICATIONS:

Ph.D. with associated degrees in agriculture and/or veterinary science.

The salary payable will be in accordance with qualifications and experience, within the New Zealand Public Service Salary Scales.

Application forms and general information are available from the New Zealand High Commission, New Zealand House, Haymarket, London SW1Y 4TQ, or the New Zealand High Commission, Commonwealth Avenue, Canberra A.C.T. 2600, Australia or the New Zealand High Commission, Suite 804, Commonwealth Building, 77 Metcalfe Street, Ottawa, Ont. K1P5L6, Canada.

When making enquiries from the Chief Migration Officer, New Zealand House, Haymarket, London SW1Y 4TQ please quote reference Imm 2/14/59.

Closing date for applications November 13th 1978.

7357(A)

LANCHESTER POLYTECHNIC

Coventry

FACULTY OF APPLIED SCIENCE

TEMPORARY LECTURER GRADE II IN MICROBIOLOGY

(This post is available for one year only)

Experience required in general microbiology, including a familiarity with basic microbiological techniques and the broad groupings of prokaryotes and eukaryotes; some experience in medical microbiology would be useful.

Applicants should possess a degree with relevant experience in teaching and/or research or professional practice in an appropriate field. A higher degree will be an advantage.

Salary: £4,101 - £6,558 p.a.

For application forms and further particulars please apply in writing enclosing a foolscap stamped and addressed envelope to the Personnel Officer, Lanchester Polytechnic, Priory Street, Coventry CV1 5FB returnable by 16th October, 1978.

7327(A)

The University of British Columbia

DEPARTMENT OF CHEMISTRY

Applications are invited from candidates with teaching and research interest in any aspect of INORGANIC CHEMISTRY including BIOLOGICAL INORGANIC CHEMISTRY for an appointment at the Assistant Professorship level.

Applications consisting of a curriculum vitae, a list of publications and the names and addresses of three referees should be sent to the undersigned before December 31, 1978. This post is open to both male and female applicants.

Professor Charles A. McDowell
Department of Chemistry
University of British Columbia
2075 Wesbrook Mall
Vancouver, British Columbia
Canada, V6T 1W5

7313(A)

SCIENTIFIC EDITOR

PHARMACEUTICAL RESEARCH AND
NEW PHARMACEUTICAL PRODUCTS

We publish Scrip World Pharmaceutical News, the leading international pharmaceutical industry weekly business magazine. Following the promotion of the present editor to a new post, we wish to appoint a scientific editor who will be responsible for reporting on international pharmaceutical research and new drug development and marketing.

QUALIFICATIONS: A good degree in pharmacy, pharmacology or biochemistry; A knowledge of one or more European language(s) would be an advantage; and experience of technical medical writing.

THE WORK: To write comprehensively about all aspects of research and marketing of new medical products around the world using a wide range of information sources including attending international conferences.

EXPERIENCE: We are looking for someone who is able to analyse and review medical data and has experience of technical writing. He or she may have worked either for a technical medical publication, in a pharmaceutical company's technical information department, or in a university or government department.

THE SALARY: Dependent on experience and qualifications: We will pay the right person a competitive salary to what he/she is currently earning.

If you would like to be considered for this key position in our team of writers please write, in confidence, giving details of yourself and your career to date to:

Dr P. J. BROWN
P. J. B. PUBLICATIONS LTD.
53a GEORGE STREET,
RICHMOND
SURREY TW9 1HE.

7290(A)

PJB

NEWCASTLE UPON TYNE POLYTECHNIC DEPARTMENT OF MATHEMATICS, STATISTICS AND COMPUTING RESEARCH ASSISTANT

To investigate 'Mathematical Modelling of Nozzle Blockage'. The successful candidate will join a research group which is active in the field of mathematical modelling and will undertake a research project concerned with the mathematical modelling of the formation of crusts on the inside of nozzles through which molten metals are being poured. Particular emphasis will be given to the problem of nozzle blockage. The project is being carried out in collaboration with the British Steel Corporation. The Research Assistant will be encouraged to register for a higher degree.

This appointment offers a salary of £3,162 to £3,555 per annum and is initially for two years.

For further particulars and application forms, returnable by Thursday, October 19, 1978, please send a stamped addressed foolscap envelope to the Staffing Officer, Newcastle upon Tyne Polytechnic, Ellison Building, Ellison Place, Newcastle upon Tyne NE1 8ST. 7325(A)

DALHOUSIE UNIVERSITY Halifax, Nova Scotia DEPARTMENT OF ANATOMY Position Open: PROFESSOR OR ASSOCIATE PROFESSOR

Applications are invited from persons with an M.D. or Ph.D. degree for the above position which will be available July 1, 1979. Experience in teaching medical Gross Anatomy and an established research program are required. Duties may also include the teaching of Anatomy to Medical, Dental, Health Professional and/or Science students and an ability to teach Neuroanatomy or Histology would be an advantage. Salary and rank commensurate with qualifications and experience. Applications, together with curriculum vitae and the names of two referees should be addressed to Dr D. G. Gwyn, Head, Department of Anatomy, Dalhousie University, Halifax, Nova Scotia, Canada B3H 4H7. 7322(A)

THE UNIVERSITY OF BIRMINGHAM DEPARTMENT OF CLINICAL GENETICS LECTURESHIP IN HUMAN GENETICS

Applications are invited for the post of Lecturer (Non-clinical scale) in the Department of Clinical Genetics.

The successful candidate will work in the Infant Development Unit of the Birmingham Maternity Hospital in the Queen Elizabeth Medical Centre, adjacent to the University campus. The Unit has teaching, research and N.H.S. service commitments primarily in the fields of clinical genetics, population genetics, chromosomal mapping, complement typing, the interpretation of pedigree data by numerical methods, and the computational problems associated with transplantation. The Unit is associated in its work with the tissue typing and regional cytogenetics laboratories, and with the Universities of Iceland and Newfoundland.

Applicants should have a postgraduate degree or, if medically qualified, membership of a Royal College. Salary Scale: Non-clinical; £3,883 to £7,754 per annum plus superannuation.

Applications naming three referees should be sent to the Assistant Registrar, the Medical School, Birmingham B15 2TJ not later than November 18, 1978, quoting reference number LEC/CG/178. Further details may be obtained from Professor J. H. Edwards, Infant Development Unit, Birmingham Maternity Hospital, Birmingham B15 2TG. 7317(A)

UNIVERSITY OF READING POSTDOCTORAL RESEARCH ASSISTANT GEOCHEMIST

required as soon as possible, for a fixed term to be determined, for research on volatiles in magmas and minerals. The project funded for three years by the N.E.R.C. is supervised by Professor D. K. Bailey and is to apply new techniques to the extraction and analysis of gases from glassy lavas, and volatile-bearing minerals, with the aim of elucidating igneous and ore-forming processes. Applicants should already have a doctorate (or be submitting a thesis) in geochemistry. Experience in experimental petrology, mass spectrometry, or gas chromatography will be an advantage. Salary £3,883 per annum plus U.S.S. superannuation. Apply quoting Ref. M.N. 48A by November 6, with curriculum vitae and the names of two referees to Assistant Bursar (Personnel), University of Reading, Whiteknights, Reading RG6 2AH. 7360(A)

UNIVERSITY OF STRATHCLYDE PROFESSOR OF FORENSIC SCIENCE

Applications are invited from suitably qualified and experienced candidates for appointment to the newly established Professorship in Forensic Science.

Applicants should have substantial experience in one or more of the major aspects of Forensic Science, and be capable of directing postgraduate teaching and research as well as contributing to casework studies.

The post will be remunerated within the professorial range for universities with superannuation benefit.

Application forms and further particulars (quoting 48/78) may be obtained from the Registrar, University of Strathclyde, Royal College Building, 204 George Street, Glasgow G1 1XW, with whom applications should be lodged by November 8, 1978. 7359(A)

BRITISH POSTGRADUATE MEDICAL FEDERATION (University of London)

Geoffrey E. Duveen Fund
FOR THE PROMOTION OF
RESEARCH IN OTOTOLOGY

Applications are invited for support from the Geoffrey E. Duveen Fund for research in aspects of Otology and Neuro-Otology. Funds, which will be available during 1979/80, may be used either for:-

(a) A Junior Fellowship, to be called the 'Geoffrey E. Duveen Fellowship', normally tenable in a School of the University or an Institute of the British Postgraduate Medical Federation for a period of one year, with a possible extension for a second year. The stipend will be within the annual range of £3,600 to £4,600.

(b) To provide financial assistance in the form of grants towards technical assistance or the expenses of appropriate research conducted in Schools of the University or Institutes of the Federation.

Applications should be sent to the Director, British Postgraduate Medical Federation, 33 Millman Street, London WC1N 3EJ, by October 31, 1978. Applicants under (a) should submit a full curriculum vitae, details of the research proposed and the names and addresses of two referees. Applicants under (b) should submit particulars of the project for which they require financial assistance and details of the cost of technical assistance or equipment required. 7354(A)

TECHNICAL UNIVERSITY
OF CRETE
ADMINISTRATIVE
COMMITTEE
ANNOUNCEMENT

The Administrative Committee of the Technical University of Crete is interested in compiling information concerning scientists who may be interested in taking a professional or research position in one of the Schools or Research Institutes of the University. It therefore invites all who may be interested in such a position, to send to the Main Office of the university (Zaimi 2, Athens TT 148, GREECE, Attention: Professor P. Theoharis) a Curriculum Vitae containing full name and address, scientific accomplishments and areas of scientific interests. In this way the Administrative Committee will be able to keep informed about the vacancies, those interested in them.

It is expected that at the beginning of the next calendar year there will be 15 new professional positions in the first three Schools and two Research Institutes, of the University. The creation of more positions for the remaining Schools and Institutes will take place gradually within a couple of years.

For more detailed information please send your inquiries to the address given above. 7362(A)

ROTHAMSTED
EXPERIMENTAL STATION
Harpenden, Herts. AL5 2JQ
ENTOMOLOGIST

required to act as co-ordinator of a team in the Entomology Department working on the integrated control of cereal pests. The appointee would also be expected to study individually the biology, behaviour and ecology of a group of natural aphid predators/parasites relevant to the integrated control studies.

Qualifications: First or upper second class honours degree in applied entomology or zoology, with at least two years' postgraduate research experience. An interest in crop husbandry and possibly taxonomy would be an asset.

Appointment in grade of Higher Scientific Officer (£4,101 to £5,448 per annum) or Senior Scientific Officer (£5,154 to £6,898 per annum), depending on experience. At least four years' relevant postgraduate experience required for Senior Scientific Officer. Non-contributory superannuation.

Apply in writing to the Secretary giving names and addresses of two referees and quoting Ref. 371 by October 27, 1978. Further details on request 7326(A)

THE UNIVERSITY
OF HULL
DEPARTMENT OF
ZOOLOGY
CHAIR OF
APPLIED ZOOLOGY

Applications are invited for the Chair in Applied Zoology tenable from January 1 or at a later date to be arranged. The Professor will take the lead in diversifying the teaching base within the Department. Applications are invited from persons who consider their scientific experience would provide the impetus for new developments into the field of Applied Zoology, which the University views in its broadest interpretation as the application of animal biology to human affairs.

Initial salary will be not less than £9,443 per annum plus F.S.S.U./U.S.S. benefits.

Applications (12 copies) naming three referees should be sent by November 6, 1978 to the Registrar, The University of Hull, Hull HU6 7RX from whom further particulars may be obtained. 7320(A)

CSIRO AUSTRALIA Plant Breeder Research Scientist or Postdoctoral Research Fellow Division of Horticultural Research

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,200 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Salinity Research

Location:

The Division has laboratories at Adelaide, South Australia (Headquarters) and Merbein, Victoria. The successful appointee will be located at the Merbein laboratory.

General:

The Division is concerned with research on a range of perennial horticultural plants (deciduous and evergreen) from both temperate and subtropical regions. Current programs include studies on the management and genetic improvement of grapevines and fruit trees and of plant viruses and plant parasitic nematodes. Research in plant physiology, biochemistry and cytology relates to photosynthesis, salinity and plant and fruit development.

Duties:

To develop and select salt tolerant crop plants in collaboration with plant physiologists. Experimentation would involve annuals as well as perennial horticultural species.

Qualifications:

A Ph.D. degree in plant breeding and genetics, or equivalent qualifications.

Salary:

Research Scientist or Senior Research Scientist: \$A14,829 to \$A21,543 p.a.

Tenure:

Either: (i) an indefinite appointment as Research Scientist; or (ii) a fixed-term appointment as Postdoctoral Research Fellow, may be negotiated. Both carry Superannuation benefits.

Applications IN DUPLICATE, stating FULL personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 491/49 should reach:

The Personnel Officer,
Australian Scientific Liaison Office,
Canberra House,
Maltravers Street,
LONDON
WC2R 3EH,

by 4th NOVEMBER 1978.

Applications in U.S.A. and Canada should be sent to:

The Counsellor (Scientific),
Embassy of Australia,
1601 Massachusetts Avenue, N.W.,
WASHINGTON D.C. 20036,
U.S.A.

7378(A)

NEW ZEALAND
Ministry of Agriculture and Fisheries

SCIENTIST

Levin Horticultural Research Centre
(Vacancy No. 5560)

Salary payable depending on qualifications and experience, up to N.Z. Dirs19642 p.a.

DUTIES:

A permanent position is available in the Ornamentals and Glasshouse Section for applied Research on Food or Ornamental Crop Production.

QUALIFICATIONS REQUIRED:

An honours degree or a higher degree in Horticulture or Science with experience in research, extension or commercial production of either Greenhouse or Nursery Crops, together with proven ability in research, communication and co-operation in a small research team.

SCIENTIST

Hastings Horticultural Research Station
(Vacancy No. 5559)

Salary payable depending on qualifications and experience up to N.Z. Dirs19642 p.a.

DUTIES:

Officer in charge, responsible for the Ministry's Research Programme on Crops for processing in the Hastings District.

QUALIFICATIONS:

Honours degree or Phd in Horticulture, Agriculture or Plant Science.

Passages and Incidental Expenses. Consideration may be given to providing assistance with passages and incidental expenses.

Applications with full details of age, experience and family should be sent to:—

The Chief Migration Officer,
New Zealand House,
Haymarket,
LONDON SW1Y 4TQ

You should quote reference Imm 2/320/15 and the Vacancy number in your application.

Closing date for application 17th November, 1978.

7336(A)

SOUTH GLAMORGAN HEALTH AUTHORITY (T)
UNIVERSITY HOSPITAL OF WALES

SCIENTIFIC OFFICER

DEPARTMENT OF HAEMATOLOGY

A graduate is required to take part in the biochemical investigation of abnormalities in iron metabolism and erythropoiesis. Duties will include the use of spectrophotometric, atomic absorption and immunoradiometric assay techniques. The person to be appointed should have an interest in developing a career in hospital laboratory medicine.

The appointment is on the Basic Grade Scientific Officer scale £2,991 to £4,899.

Application forms from: Personnel Department, University Hospital of Wales, Heath Park, Cardiff. Tel: 0222 755944.

Further particulars may be obtained from: Mr B. Jones, Department of Haematology, University Hospital of Wales, Heath Park, Cardiff. Tel. (0222) 755944 ext. 2372. Closing Date: October 20, 1978.

7361(A)

ST GEORGE'S HOSPITAL
MEDICAL SCHOOL
(University of London)
TECHNICIAN 5

Experienced Technician 5 required to work mainly as personal assistant to Professor P. N. Dilly, Department of Structural Biology, without close supervision in all aspects of the fields of transmission and scanning electron microscopy and photography. A knowledge of histological techniques would be an advantage. Emoluments range £3,461 to £3,995 per annum. Application forms available from the Establishment Officer, St. George's Hospital Medical School, Cranmer Terrace, London SW17 0RE.

7334(A)

THE UNIVERSITY OF
PAPUA NEW GUINEA
(Port Moresby)

Applications are invited for the post of LECTURER IN PHYSIOLOGY in the DEPARTMENT OF HUMAN BIOLOGY tenable from January 1979. The Department of Human Biology teaches preclinical basic medical sciences to second and third year medical students. The Department teaches an integrated programme of courses comprising human structure, physiology and neurophysiology, biochemistry and pharmacology. Applicants should have a higher degree in science or medicine and have experience in teaching physiology to medical students. Experience in teaching in a developing country would be an advantage. Salary: Lecturer K9,048 to K14,218 per annum (£1 Sterling equals K1.35). In addition, an allowance of K1,300 if single is payable. An extra K1,000 Marriage Allowance and K156 per child Child Allowance may also be payable. Conditions include provision of housing; annual leave fares; study leave and F.S.S.U. In some cases it may be possible to make an appointment on secondment. Detailed applications (two copies) with curriculum vitae and also naming three referees, with a small photograph, to be sent direct to the Secretary, University of Papua New Guinea, Box 4820, Papua New Guinea by November 3, 1978. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

7335(A)

LONDON SCHOOL OF
HYGIENE AND
TROPICAL MEDICINE
(University of London)

Keppel Street, WC1E 7HT
CLINICAL NUTRITION AND
METABOLISM UNIT
4 St Pancras Way, NW1 2PE
A MEDICAL LABORATORY
SCIENTIFIC OFFICER

is required up to September 30, 1981 to work on the regulation of protein synthesis and breakdown in rats and man. Candidates should preferably be graduates in biochemistry or hold equivalent qualifications. Inclusive salary, depending on qualifications and experience, rises to a maximum of £4,284. Applications, consisting of full career and education details and naming two referees, should be sent to Dr P. J. Garlick at the Unit.

7332(A)

LONDON SCHOOL OF
HYGIENE AND
TROPICAL MEDICINE
(University of London)

Keppel Street, WC1E 7HT
DEPARTMENT OF
MEDICAL PROTOZOLOGY
LECTURESHIP

A new post has been created for the study of the immunology of protozoal infections of mucous surfaces. Applications are invited from suitably qualified candidates.

The person appointed will be expected to participate in the general teaching of the Department and the supervision of higher degree students as well as to undertake an active research programme. An ability to attract outside support for the research work will be an advantage. Salary will be on the "interim" scale for lecturers, ranging from £3,883 to £7,754 plus £450 London Allowance. Further details of the appointment may be obtained from Professor W. H. R. Lumsden. Application forms may be obtained from the Dean at the School and should be returned to him by November 16, 1978.

7333(A)

QUEEN MARY COLLEGE
(University of London)
DEPARTMENT OF PLANT
BIOLOGY AND MICROBIOLOGY
A Grade 3 and a Grade 5
TEACHING/RESEARCH
TECHNICIAN

required to work in a Molecular Genetics group headed by Professor E. A. Bevan. The main research interests of the group are concerned with viral infections and cytoplasmic organelle genetics in yeast. The teaching duties involve assisting in the preparation of laboratory classes, especially those dealing with aspects of Microbial and Molecular Genetics. Some experience in handling micro-organisms, biochemical separation including ultra-centrifugation techniques, would be useful.

Minimum qualifications, Grade 3 O.N.C. and Grade H.N.C. or equivalent. Salary scales £2,688 to £3,060 per annum and £3,186 to £3,720 per annum respectively and in both cases plus £465 per annum London Weighting. 5-day week, 4 weeks annual leave plus a week at Christmas and Easter which includes the public holidays.

Letters only to Assistant Secretary (Establishment), PB/DM, Queen Mary College, Mile End Road, London E1 4NS, stating age, experience, qualifications and which post applied for. Closing date: October 11, 1978.

7375(A)

THE BRITISH COUNCIL
INVITES APPLICATIONS FOR
THE FOLLOWING POST
TEACHER OF
SILVICULTURE
TEACHER OF BOTANY
(Iran)

Higher School of Natural Resources, Gorgan. To teach up to B.Sc. level, give practical instruction, set and mark examinations, assist with curriculum development. Degree plus postgraduate diploma or preferably M.Sc. in forestry or related subjects and at least two years' relevant teaching experience. Candidates, men only, should be single or married with a maximum of two children under five.

Salary: £5,750 to £7,048 per annum tax-free.

Benefits: Personal and children's allowances; free furnished accommodation; baggage allowance. One year contract, renewable.

Return fares are paid. Local contracts are guaranteed by the British Council. Please write briefly stating qualifications and length of appropriate experience, quoting reference number 77 HO 40 & 42, and title of post, for further details and application form to The British Council, 65 Davies Street, London W1Y 2AA.

7346(A)

THE GRASSLAND
RESEARCH INSTITUTE
Hurley, Maidenhead
Berkshire
SYSTEMS ANALYSIS
IN AGRICULTURE

The Institute is seeking an officer to develop and apply the techniques of systems analysis and mathematical modelling to biological and agricultural problems.

Applications are invited from scientists with a basic training in mathematics, statistics and computer programming or the physical sciences. Computing facilities include access to ICL 4/72 and IBM 370 machines.

The Grassland Research Institute is grant aided by the Agricultural Research Council and is associated with the University of Reading.

The appointment will be made in the Higher Scientific Officer or Senior Scientific Officer grade.

SALARY: H.S.O £4,101 to £5,488; S.S.O £3,154 to £6,898, grade and starting salary will depend on experience.

QUALIFICATIONS: A First or Upper second class honours degree or the equivalent in an appropriate subject and at least two years' relevant post-qualifying experience.

A minimum of four years' relevant post-qualifying experience is required for appointment to the S.S.O grade.

Applications to the Secretary with curricula vitae and the names of three referees by October 27, 1978.

7376(A)

WELSH NATIONAL SCHOOL
OF MEDICINE
(University of Wales)
K.R.U.F. INSTITUTE OF
RENAL DISEASE
CARDIFF ROYAL INFIRMARY
SEROLOGIST/
IMMUNOLOGIST

required for a 2-year appointment funded by the M.R.C., to study antibodies in kidney transplant patients. Knowledge of tissue typing techniques would be an advantage.

Commencing salary could be up to £4,631 per annum for suitably qualified applicants.

Application forms available from the Registrar and Secretary, Welsh National School of Medicine, Health Park, Cardiff (quoting Ref. No M6/7/14). Further information available from Mr J. Salaman, F.R.C.S (Tel: Cardiff 492233.) 7365(A)

UNIVERSITY OF SYDNEY
LECTURESHIP IN
VETERINARY PATHOLOGY

Appointee to be responsible for teaching research and diagnostic services in the field of Veterinary Clinical Pathology and will be located in Sydney Veterinary qualifications or equivalent required. Experience in clinical or practical pathology desirable.

The position may be filled either on a tenured basis, or in the first instance, for a probationary period of three years with the possibility of tenure being given during or shortly after that period, or in certain cases return fares.

Salary Range: \$A15,179 to \$A19,940 per annum.

Applications including curriculum vitae, list of publications and names of three referees by October 31, 1978 to the Registrar, University of Sydney, N.S.W. 2006, Australia, from whom further information available. Information also available from Association of Commonwealth Universities (Apsu), 36 Gordon Square, London WC1H 0PF. 7353(A)

CSIRO AUSTRALIA Research Scientist Division of Building Research Hihett, Victoria

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,200 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Microbiology/Mycology

Duties:

To form part of the Division's multidisciplinary Conservation and Biodegradation Section by conducting research on both fundamental and applied levels into the fungal decay of preservative-treated and untreated wood and other diverse structural materials. The work includes the development of control mechanisms, chemical, physical or biological; the physiology and ecology of biodeteriogens and their mechanisms of degradation; and some fungal taxonomy. The candidate should have a sound mycological training and background, and will be expected to work both as an individual researcher and as a member of a research team.

Qualifications:

A PhD degree in mycology/microbiology, agricultural science, biochemistry, or other relevant discipline and previous research ability. Preferably the applicant should possess a sound working knowledge of biochemistry as an addition to his major area or expertise in mycology.

Salary:

Research Scientist or Senior Research Scientist: \$A14,829—\$A21,543 p.a.

Tenure:

Indefinite with superannuation.

Applications IN DUPLICATE, stating FULL personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 390/627 should reach:—

The Personnel Officer,
Australian Scientific Liaison Office,
Canberra House,
Maltravers Street,
LONDON WC2R 3EH

by 4th November 1978.

Applications in U.S.A. and Canada should be sent to:—

The Counsellor (Scientific),
Embassy of Australia,
1601 Massachusetts Avenue, N.W.,
WASHINGTON, D.C., 20036, U.S.A.

7377(A)

AWARDS

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF BIOCHEMISTRY

S.R.C. C.A.S.E AWARD

"Sillcos and related materials as beer stabilisers".

Cooperating Body. Unilever Research, Port Sunlight.

Applications are invited from suitably qualified biochemists and chemists by November 20, to Professor J. S. Hough, Biochemistry Department, The University, P.O. Box 363, Birmingham B15 2TT. Commencement date in 1979 is subject to discussion. 7288(N)

M.R.C. CLINICAL RESEARCH CENTRE (Northwick Park Hospital)

Watford Road, Harrow, Middx. HA1 3UJ

The Division of Immunological Medicine (Head Dr. G.L. Asherson) require a medical or recent post-doctoral person interested in studying the cellular defects in immune deficiency disease and in particular hypogammaglobulinaemia. The appointment is for three years only and the salary is on NHS registrar/sen. reg. scale or on university lecturer scale depending upon qualifications.

Application form obtainable from Mrs. J. Tucker-Bell by October 21st. Please quote ref. 118/1/4209. 7339(A)

THE OPEN UNIVERSITY



Lecturers in Science Biology

We have two vacancies: those appointed will be involved in the preparation and maintenance of the discipline's 2nd and 3rd level courses in one or more of the areas of Ecology, Mammalian Physiology, and Molecular Biology/Biochemistry. They will also be expected to take an active interest in the development of the discipline's future offerings in the field of continuing education, and to undertake research—either independently, or with one of the existing research groups. For these posts, candidates are likely to be under 30, with teaching experience and a good research record.

Ref: 3662

Earth Sciences

We have two vacancies: the successful applicants will take part in the remaking of the 2nd level courses in Geology or the Earth's Physical Resources, and may have research interests in any branch of the discipline. They will be expected to pursue those research interests vigorously.

Ref: 3663

Physics

There is one vacancy in this discipline: the person appointed will assist in the preparation of appropriate undergraduate courses, using multi-media teaching methods, and will also be expected to undertake research—either independently or with colleagues.

Ref: 3660

Applicants for all posts should be able to demonstrate an ability to write clearly and easily and an interest in distance teaching. All five posts are tenured: salary will be on the scale £3,883—£7,754 (under review and there are U.S.A. and other benefits).

Application forms and further particulars are available, by postcard request please, from The Recruitment Office (JD3663/00), The Open University, P.O. Box 75, Walton Hall, Milton Keynes, MK7 6AL, or telephone Milton Keynes 63868: 24 hour answering service.

PLEASE STATE THE DISCIPLINE IN WHICH YOU ARE INTERESTED

Closing date for applications: 20th October 1978. 7315(A)

FORTH RIVER PURIFICATION BOARD

PHYSICAL OCEANOGRAPHER

Applications are invited for the post of Physical Oceanographer with the Board's Estuary Survey Section.

The successful applicant will join a small team of specialists working on water quality problems within the Firth of Forth. Ideally candidates should have a sufficient understanding of numerical analysis and estuarine processes to be able to improve the Board's existing steady state model and to develop a time varying model of the Forth Estuary. The Forth Estuary is of particular interest as it demonstrates many of the transient features associated with a partially mixed estuary. Facilities include direct access, via a terminal, to 2M bytes of core store on a local University computer system and use of the Board's 60 foot survey vessel. Apart from modelling studies, duties will include involvement in surveys of outfall sites and the hydrography of inshore areas.

The post is superannuable and subject to N.J.C. conditions of service. Salary will be in the range £4,368 to £5,403 inclusive of £312 supplement, with placing according to age, qualifications and experience.

Applications should include the names of two referees and be submitted not later than Friday, October 20, to:

The River Inspector,
Colinton Dell House,
West Mill Road,
EDINBURGH EH13 0PH.

7308(A)

MEDICAL RESEARCH COUNCIL ELECTRON MICROSCOPIST

Applications are invited from graduates with a degree in Medicine, or Biological Sciences to develop a research programme into the mechanism of action of toxic substances. The successful candidate will be responsible for leading a group consisting of one other scientist (to be appointed) and two technicians together with research students and visiting scientists. He will also be responsible for promoting collaborative research with other members of the unit working Toxicological problems, either in the whole animal or in isolated cells cultured *in vitro*.

Salary on M.R.C. scientific staff scales (non-clinical) depending on age and experience.

Apply in writing enclosing a full curriculum vitae and giving the name of two referees to the Director, Toxicology Unit, Medical Research Council Laboratories, Woodmansterne Road, Carshalton, Surrey SM5 4EF.

MRC
Medical Research Council

7272(A)

COMMONWEALTH AGRICULTURAL BUREAUX Vacancy for SCIENTIFIC INFORMATION OFFICER at the COMMONWEALTH INSTITUTE OF ENTOMOLOGY 56 Queen's Gate, London SW7 5JR

Duties: Mainly preparing abstracts in English for the journal 'Review of Applied Entomology' which covers the world of scientific literature on injurious and useful insects. Either one full-time or two part-time appointments would be considered.

Qualifications: Graduate required with ability to read Russian or German and preferably some knowledge of biology or agriculture; knowledge of other Slavonic or of Scandinavian languages would also be useful. The ability to write good, concise English is essential.

Salary: In the scale £2,839 to £5,448 per annum plus Inner London Weighting of £465 per annum, plus a compensatory allowance (taxable but not superannuable) of 3.3 per cent to offset personal contribution to Superannuation Scheme; part-time appointments pro-rata. Starting salary according to qualifications, experience and age.

Application forms and full particulars from the Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN, to whom completed applications (in duplicate) should be sent by October 28, 1978.

7318(A)

ST. THOMAS' HEALTH DISTRICT (TEACHING) London SE1 MEDICAL LABORATORY SCIENTIFIC OFFICER (Grade subject to qualifications and experience) DEPARTMENT OF CHEMICAL PATHOLOGY AND METABOLIC DISORDERS ST. THOMAS' HOSPITAL

A post is available in the Supra Regional Hormones Laboratory, which will involve work on both protein and steroid hormones. Previous experience in radioimmunoassay would be an advantage, but is not essential.

The successful candidate will have the opportunity of pursuing further relevant qualifications.

For application form and job description, contact Ms J. Frost, Personnel Officer, St. Thomas' Hospital, London SE1 7EH, quoting ref. SRHL. Closing date October 20, 1978.

7289(A)

BIOCHEMIST—MOLECULAR BIOLOGIST

The Department of Biological Sciences, Carnegie-Mellon University invites applications from molecular biologists whose research interests lie in the area of nucleic acid structure and/or function. Candidates should have postdoctoral experience. The position is at the assistant professor level, although higher appointments will be considered. Duties include teaching at the undergraduate and graduate level as well as maintaining an active research programme. Applicants should forward their curriculum vitae, three or more letters of reference and a brief résumé of research plans to Search Committee (2), Department of Biological Sciences, Carnegie-Mellon University, 4400 Fifth Ave., Pittsburgh, PA 15213. *Carnegie-Mellon University is an Equal Opportunity/Affirmative Action Employer.*

7349(A)

INSTITUTE OF ORTHOPAEDICS (University of London), Research Assistant, Honours Graduate in Biology to participate in research into human hip disease. Suitable for M.Phil./Ph.D. Three year Arthritis and Rheumatism Council grant. Information from and applications to the Secretary, Institute of Orthopaedics, Brockley Hill, Stanmore, Middlesex HA7 4LP, with two referees.

7309(A)

POSITION OPEN

A postdoctoral position is available for a period of one to three years to study mechanisms of action of peptides (substance P, somatostatin, endorphins) in mammalian spinal pain pathways using predominantly electrophysiological techniques. Send curriculum vitae and three references by January 1, 1979 to M. Randic, Department of Veterinary Anatomy, Pharmacology and Physiology, Iowa State University of Science and Technology, Ames, Iowa 50011. An Affirmative Action/Equal Opportunity Employer.

7350(A)

UNIVERSITY COLLEGE OF NORTH WALES, BANGOR TECHNICIAN GRADE 4

£2,955 to £3,402 per annum

Applications are invited for the post of Histology Technician in the Department of Zoology.

The main duties will be the preparation and sectioning of material for examination in the light microscope together with some assistance in the laboratories.

Qualifications required are O.N.C. or an equivalent qualification.

Applications (two copies), giving details of age, education and experience, together with the names and addresses of two referees should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, by not later than October 16, 1978.

7358(A)

TECHNICIAN GRADE 2B

required for the teaching laboratories of the Biophysics Department. This is an interesting post concerned with the teaching of a wide range of courses. Other duties include help with the annual unit and helping academic staff with research. Salary on scale £2,994 per annum rising to £3,345 per annum inclusive. Four weeks annual holiday. College contributory pension scheme. Apply in writing with full details to: The Head Clerk, King's College London, Strand, WC2R 2LS. Ref. 204016/N. 7283(A)

QUEEN ELIZABETH COLLEGE

Kensington

(University of London)

GRADE IV RESEARCH

TECHNICIAN

Required to maintain and operate a Kratos AEI MS25/DS508 gas chromatograph-mass spectrometer with computer data processing. This new instrument will provide a GC/MS service under the University of London Inter-collegiate Research Service, and will specialise in capillary column GC and chemical ionisation MS. The unit at Q.E.C. already possesses an MS30/DS50 and the successful candidate will be expected to operate this instrument as well, and to carry out duties assigned by the Technician in charge of the unit. Experience in mass spectrometry is desirable but not essential and necessary on-site training is available.

Salary within range £3,420 to £3,567, inclusive of London Allowance.

For application forms contact Mrs G. M. Howard, Tel. 01-937 5411, Ext. 499, Queen Elizabeth College, Campden Hill Road, London W8 7AH. 7284(A)

THE MIDDLESEX HOSPITAL MEDICAL SCHOOL

(University of London)

RESEARCH ASSISTANT

A group engaged in research on the metabolism and functions of endogenous peptides and steroids concerned with the control of blood vessels and of water and electrolyte metabolism requires a graduate in chemistry, biochemistry, medicine or related fields. The work, involving organ perfusions, enzyme assays and radioimmunoassays, will be suitable for a higher degree thesis. Starting salary £3,663 per annum inclusive of London Allowance. Applications with full curriculum vitae and names and addresses of two referees should be sent by October 26, to Dr J. D. H. Slater, Institute of Clinical Science, The Middlesex Hospital Medical School, London W1P 7PN. 7285(A)



CHAIR OF GEOLOGY

Applications are invited for a further Chair of Geology which is to be established during the academic year 1978/79. Salary within the professional range. Applicants may have teaching and research interests in any field of study appropriate to a University Department of Geology. Further particulars may be obtained from the Secretary and Registrar, The University, Southampton SO9 5NH, and applications (10 copies from applicants in the United Kingdom) must be submitted before November 5, 1978. 7293(A)

CSIRO

AUSTRALIA

Research Scientist or Post Doctoral

Research Fellow

Division of Fisheries & Oceanography

Perth, Western Australia

CSIRO has a broad charter for research into primary and secondary industry areas. The Organization has approximately 7,000 employees—2,300 of whom are research and professional scientists—located in Divisions and Sections throughout Australia.

Field: Biological Oceanography

General:

The Division is Australia's principal marine research institution with about 70 scientists investigating the physical, chemical and biological features including fisheries of the oceans around Australia. Headquarters of the Divisions are at Cronulla, Sydney, with regional laboratories in Perth, Brisbane and a field laboratory in Karumba. On-line access to a Cyber 76 computer is available. Two well-equipped medium sized chartered vessels are available for oceanographical research and there are several smaller CSIRO vessels.

Duties:

To initiate and carry out research in coastal marine ecology, as part of a research team investigating the production kinetics of a limestone reef.

Qualifications:

Marine ecologist with experience in community ecology of reef ecosystems. Some special knowledge of nutrient cycling and fisheries within such systems would be an advantage. Minimum qualifications are a Ph.D. or equivalent qualification in a relevant field of marine biological research, together with appropriate postdoctoral experience.

Salary:

Dependent upon experience within the ranges: Research Scientist: \$A14,829 — \$A18,177 p.a. or Senior Research Scientist: \$A18,819 — \$A21,543 p.a.

Tenure:

An indefinite appointment or a fixed-term appointment of 3 to 5 years may be negotiated. Both carry Superannuation benefits.

Applications IN DUPLICATE, stating FULL personal and professional details, the names and addresses of at least two professional referees, and QUOTING REFERENCE NUMBER 320/604 should reach:—

The Personnel Officer, Australian Scientific Liaison Office, Canberra House, Maltravers Street, LONDON WC2R 3EH. by 4th November 1978.

Applications in U.S.A. and Canada should be sent to:—

The Counsellor (Scientific), Embassy of Australia, 1601 Massachusetts Avenue, N.W., WASHINGTON, D.C., 20036, U.S.A. 7379(A)

Careers in Drug Metabolism Greenford, Middlesex

Two vacancies have arisen in our laboratories for appropriately qualified scientists to be responsible for the development and use of analytical procedures for measuring drug levels in biological materials.

The work is concerned with the study of the absorption, distribution and metabolism of existing and new therapeutic agents. One of the above vacancies would suit a graduate with two to three years relevant experience, possibly with a post-graduate qualification, although this is not essential. The second vacancy would be suitable for a person who has recently qualified. In both cases candidates should hold good first degrees in relevant scientific disciplines and have an interest in bio-analytical methods.

The Company offers excellent conditions of employment including pension and bonus schemes, subsidised cafeteria and a sports and social club.

Please write or telephone for an application form to:

Miss E. M. Butler
Secretary to Senior Personnel Officer
Glaxo-Allenburys Research (Greenford) Limited
Greenford, Middlesex.
Quoting Ref. 241
01-422 3434, Ext. 11

7363(A)

Glaxo-Allenburys Research (Greenford) Limited

Director of Research

A major French pharmaceutical group is looking for a top-flight scientist, with biological, medical and pharmaceutical qualifications. To be located in Paris, the director of research will be entrusted with determining, in co-ordination with the general management, the group's research policy with the application of that policy and with participating in general policy decisions, including licensing to other groups.

Salary will be in accordance with qualifications and with the position offered.

Please send CV, recent photograph and current salary under ref. 1054 N.

All applications will be treated with the highest confidentiality by:

BERNARD KRIEF CONSULTANTS

1, rue Danton 75263 Paris Cedex 06

7306(A)

SYMPOSIA—(continued on page xxiv)

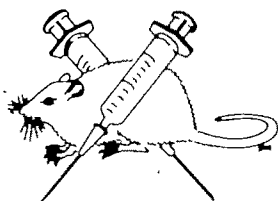


THE REDUCTION AND PREVENTION OF SUFFERING IN ANIMAL EXPERIMENTS

A SYMPOSIUM

To be held on 1st NOVEMBER 1978
at the Meeting Room, Zoological Society, Regent's Park, London NW1
Tickets—£5 00 including refreshments and buffet lunch
Further details from: Richard Ealing, RSPCA, Causeway,
Horsham, Sussex Tel Horsham 64181 extn 78

7296(M)



Simon Fraser University

DIRECTOR MASTER OF NATURAL RESOURCE MANAGE- MENT PROGRAM

A Director is required for the Master of Natural Resource Management Program which has been established at Simon Fraser University. The new program, which will admit its first students in September, 1979, consists of two years of graduate work, and is designed for recent graduates and experienced professionals. The Director will co-ordinate instructional and research programs from varied disciplines and departments.

The Director should have an established academic and professional reputation in one of the disciplines related to resource management and will be offered a permanent appointment jointly between the program and an appropriate department.

The preferred date of appointment is January, 1979, but later starting dates can be negotiated. Salary and rank will be dependent upon qualifications. Applicants should send curriculum vitae and the names of three referees to:

Dr T. W. Calvert

Dean,
Faculty of Interdisciplinary Studies,
SIMON FRASER UNIVERSITY
Burnaby, B.C., Canada
V5A 1S6 7312(A)

UNIVERSITY OF BRISTOL DEPARTMENT OF CHILD HEALTH RESEARCH ASSISTANT

Postdoctoral scientist or equivalent with experience in electron microscopy, immunology and/or autoradiography to work on immediate hypersensitivity in the lung. Starting salary up to £4,382 per annum. Applications with a curriculum vitae and two referees to Dr F. Carswell, from whom further information obtainable, Department of Child Health, Children's Hospital, Bristol BS2 8BJ.

7310(A)

WELSH NATIONAL SCHOOL OF MEDICINE (University of Wales) DEPARTMENT OF HAEMATOLOGY RESEARCH OFFICER

A graduate in biological or natural science is required to take part in an investigation of blood cell formation in haematological disorders.

The study involves analysis of the kinetics of iron metabolism and a knowledge of mathematics/computing would be an advantage.

The appointment is for two years and will be made on the Research and Analogous Staff Scale IB, £3,384 to £4,882 (under review).

Further particulars may be obtained from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff, to whom applications, in the form of a brief curriculum vitae with the names and addresses of two referees, should be submitted within two weeks of the date of this advertisement, quoting Ref. No H24/2/21. The Department may be visited by arrangement with Dr I. Cavill (0222 755944, extension 2365) 7366(A)

IMPERIAL COLLEGE FIELD STATION ZOOLOGY DEPARTMENT

A Research Assistant is required for an A.R.C.-sponsored project on movement and metabolism of systemic pesticides in relation to plant-parasitic nematodes. The work will involve use of radiotracer techniques and maintenance of experimental plants in controlled environmental conditions. Experience of work with nematodes is not essential. Salary in the IB range £3,384 to £4,882 per annum, plus U.S.S.

Applications stating age, qualifications, experience and names and addresses of two referees to: Dr D. J. Wright, Imperial College Field Station, Sunninghill, Ascot, Berkshire, from whom further details may be obtained 7352(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF PHARMACOLOGY AND THERAPEUTICS

Applications are invited for the post of postdoctoral Senior Research Assistant to work in a multi-disciplinary team studying drugs used in tropical diseases. The work will involve quantification, chemical synthesis and structural determination of drug metabolites.

The project is financed for up to three years, and the initial salary will be in the range £3,883 to £4,631 per annum.

Applications, together with the names of two referees, should be received not later than October 20, 1978, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref. RV/941/N. 7287(A)

UNIVERSITY COLLEGE LONDON DEPARTMENT OF PHYSIOLOGY LABORATORY TECHNICIAN GRADE 3

A vacancy exists for a laboratory technician to work with a small group investigating the relation between mechanical and biochemical events in muscle fibres. Starting salary £2,684 to £2,760 plus £465 London Weighting. Supported by a five-year grant from the M.R.C. Application form from Personnel Officer (Technical Staff FF4), University College London, Gower Street, WC1E 6BT.

7355(A)

FELLOWSHIPS—(continued on page xxiv)

IMPERIAL CANCER RESEARCH FUND RESEARCH FELLOWSHIP

A Postdoctoral Fellow required as soon as possible for an appointment of two years with possible extension to three years. The work will be on the mechanisms by which host cells control the expression of tumour-inducing viruses. Familiarity with nucleic acid hybridisation and allied techniques would be an advantage but is not essential. Salary with entry according to qualifications and experience within range £4,815 to £6,180 inclusive.

Applications with curriculum vitae and names of two referees, should be sent to the Secretary, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2A 3PX by November 3, 1978, quoting reference 294/78.

7297(E)

RAMSAY MEMORIAL FELLOWSHIP FOR CHEMICAL RESEARCH.

The Trustees will consider in February 1979 applications for a British (General) Fellowship, normally tenable in the United Kingdom for two years, for candidates with experience of postdoctoral research. Value of the Fellowship will normally be parallel with lower part of Lecturer scale for British Universities, plus expenses grant of up to £100 per annum. May be superannuable. The person elected will normally be required to take up the Fellowship on or about October 1, 1979. Particulars from Joint Honorary Secretaries, Ramsay Memorial Fellowships Trust, University College London, Gower Street, London WC1E 6BT. Completed application forms must be received by December 1, 1978.

7316(E)

A POSTDOCTORAL FELLOWSHIP

is now available to study the mechanism of benzene toxicity. Current investigations are aimed at understanding the relationship between the hematological/bone marrow effects of benzene and benzene metabolism (see Life Sci. 21, 1709, 1977). This is an opportunity to train in biochemical toxicology. The stipend is \$10,000.

Interested parties should write to Dr Robert Snyder, Dept. of Pharmacology, Thomas Jefferson University, Philadelphia, Pa., U.S.A. 19107. 7369(E)

UNIVERSITY OF BRISTOL POSTDOCTORATE RESEARCH FELLOWSHIP

Applications are invited for a post-doctorate fellowship established to investigate the synaptic mechanisms regulating the peptidergic neurones of the magnocellular nuclei of the rat hypothalamus. Previous experience of electrophysiological recording from the CNS is required, and some expertise in the application of iontophoretic methods is desirable.

The appointment is funded by the Medical Research Council for two years from October 1, 1978. Salary will be determined by age and experience (minimum £4,382 per annum at age 26—scale under review).

Applications giving details of age, qualifications and experience, together with the names of two referees, should be sent by October 16 to Dr D. W. Lincoln, The Medical School, University Walk, Bristol BS8 1TD, from whom further particulars are available (Bristol 24161, ext. 557).

7241(E)

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF GEOLOGICAL SCIENCES RESEARCH FELLOWSHIP— STABLE ISOTOPES IN GROUNDWATER

Applications are invited for the above post to carry out the measurement and interpretation of the stable isotopes of oxygen and deuterium in precipitation in the soil profile and in modern groundwaters. The work is supported by a research grant from the Natural Environmental Research Council.

Applicants should have a good honours degree in either Geology, Chemistry or Physics and preferably a Ph.D. Research experience related to isotope studies would be an advantage.

Salary in the range £3,883 to £6,555, plus superannuation. The starting point will be dependent on age and experience, with a maximum of £4,382. The post is tenable until September 30, 1981. Further particulars may be obtained from the Assistant Registrar, Science and Engineering, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (three copies) including full curriculum vitae, and naming three referees should be sent by Monday, October 23, 1978. Please quote ref: NG9.

7356(E)

POSTDOCTORAL FELLOWSHIP

The laboratory is engaged in full-time research (primarily biochemical) on the structure and function of microtubules (see J. Cell Biol. 73, 492 and Biochemistry 16, 2189). The candidate must have received the doctor's degree within three years, and may not be a citizen of the U.S.A. Enquiries, accompanied by a curriculum vitae and references, should be sent to: Dr Martin Flavin, Lab. of Cell Biology, NHLBI, National Institutes of Health, Bethesda, Md. 20014, USA.

7294(E)

THE UNIVERSITY OF BIRMINGHAM DEPARTMENT OF PHYSIOLOGY RESEARCH FELLOWSHIP

Applications are invited for a Research Fellowship working on a five year M.R.C.-funded project in the Department of Physiology. Applicants should have a degree in Science or Medicine and experience in Neurophysiological Research. The Fellow will study motor control in man and primates by methods that involve the application of engineering methods to physiological systems. The project is directed by Dr P. M. H. Rack from whom further information may be obtained.

Salary will be on the Research Scale 1A (£3,883 to £6,555 plus superannuation) according to age and experience. The appointment will date from January 1, 1979 or as soon thereafter as may be arranged.

Applications with curriculum vitae and names of two referees should be sent to the Assistant Registrar, The Medical School, Birmingham B15 2TT by November 24, 1978. Please quote reference RF/Phy/378.

7291(E)

POSITIONS WANTED

BIOCHEMISTRY TECHNOLOGIST, A.I.M.L.S.

25, with excellent academic record, seeks position in continental Europe, Australasia or North America. Prefer non-clinical field (e.g. research, industrial, publishing). Box No. 7265(B), c/o Macmillan Journals Ltd., 3 Dyers Buildings, London EC1N 2NR.

7265(B)

UNIVERSITY OF AUCKLAND NEW ZEALAND UNIVERSITY POSTDOCTORAL FELLOWSHIPS 1979/1980 AWARDS

Applications are invited for Fellowships to begin during the period March 1, 1979, to February 28, 1980. The Fellowships are awarded for the purpose of carrying out advanced research. Successful applicants may be expected to participate in limited teaching activities. Conditions of Appointment and application forms to be completed by candidates are available from New Zealand and Australian universities, from the Assistant Registrar (Academic Appointments), University of Auckland, Private Bag, Auckland, New Zealand, or from Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications will be accepted at any time up to **OCTOBER 31, 1978.**

The Fellowships are open to graduates of universities other than the University of Auckland; and holders of a Bachelors or Masters degree from the University of Auckland who have also completed a Ph.D. degree at any university other than the University of Auckland. Prospective candidates should note that these Fellowships are NOT open to applicants whose Ph.D. degree was completed at the University of Auckland. To be eligible for a Fellowship, candidates must have been awarded the degree of Doctor of Philosophy, or have completed the requirements for such an award, or hold some other appropriate academic qualification. The Awards are primarily intended for candidates who have held a Ph.D. for not more than four years at the time of application, but in exceptional circumstances other candidates may be considered. The total value of the Fellowships will normally be based on a monthly allowance of NZ\$700 plus, where applicable, actual approved fares for the Fellow only. Fellows should expect to pay tax on the monthly allowance.

The Fellowships are tenable for up to 12 months and have been allocated to the following research groups. Prospective candidates seeking further information should write directly to the member of staff named in the University.

Anthropology (Professor R. C. Green). Topic: Melanesian cultural history programme. Candidates in ethnohistory, historical linguistics, ethnobotany or applied science in archaeology.

Anthropology (Associate-Professor A. Hooper). Topic: Tokelau studies. Biochemistry (Professor A. G. C. Renwick). Topic: The biochemistry of glycoprotein hormone receptor interactions.

Cell Biology (Professor R. E. F. Matthews). Topic: Plant virology.

Cell Biology (Dr H. E. D. Lane/Professor P. L. Bergquist). Topic: Plasmid-specified proteins in *E. coli* minicells.

Chemistry (Professor R. C. Cambie). Topic: The utilisation of New Zealand natural products.

English (Associate-Professor MacD. P. Jackson). Topic: New Zealand literature.

Electrical Engineering—Radio Research Centre (Professor H. A. Whale). Topic: Ionospheric propagation, upper atmosphere physics and communications research.

History (Professor P. N. Tarling). Topic: National-international linkages in military occupation and post independence administration, planning and development in East and South East Asia.

History (Professor M. P. K. Sorrenson). Topic: Social, economic and political history of New Zealand.

Mathematics (Professor J. C. Butcher). Topic: Numerical solution of ordinary differential equations.

Physics (Professor A. R. Poletti). Topic: Experimental nuclear physics.

Psychology (Professor M. C. Corballis). Topic: Cognitive psychology.

Zoology (Dr B. A. Foster). Topic: Settlement and recruitment of the larvae of marine animals.

7364(E)

CYSTIC FIBROSIS RESEARCH FELLOWSHIPS

The Cystic Fibrosis Research Trust is anxious to encourage research into the problems of Cystic Fibrosis by offering special grants to medical and scientific departments and to establish Fellowships in this area of research. The Trustees would be specially interested in supporting a Fellowship in a suitable department to an individual with considerable research training especially in fields likely to lead to further knowledge of the basic abnormality in this condition. The Fellowship would ordinarily be held for three years and applications must be sponsored by the Head of the department who would be willing to provide facilities. In the first instance twelve copies of a two-page summary of the proposal should be provided, together with a curriculum vitae, a brief estimate of the anticipated costs of the Fellowship, apart from salary, should be indicated.

Applications should be addressed to Mr R. Tucker, Executive Director, C.F. Research Trust, 5 Blyth Road, Bromley, Kent BR1 3RS. Closing date: November 30, 1978.

7371(E)

FELLOWSHIPS

BRITISH/IRANIAN
SCIENTIFIC EXCHANGE
SCHEME

To stimulate Irano/British collaboration in science and technology, the Iranian Ministry of Science and Higher Education and the British Council have launched an exchange scheme, which began in April 1977. British visitors, ideally science or technologists with active research programmes established in a research institution, will spend between one and ten months participating in research or field studies, or exploring prospects for co-operative work with Iranian colleagues. Applicants who already have contacts with Iran will be given preference. The closing date for this year will be December 31. For further details and an application form, please write or telephone to Dr J. C. Viney, Higher Education and Science Department, the British Council, 10 Spring Gardens, London SW1A 2BN (telephone 01-930 8466, extension 2609). 7298(T)

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APPOINTMENTS WANTED

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Telephone: 01-831 6901

Washington Office:
711 NATIONAL PRESS BUILDING
WASHINGTON DC 20045 USA
Telephone: (202) 737 2355

SYMPOSIA

A Symposium on "Long-term
effects of Neuroleptics"

will be held in Monte-Carlo, Principauté de Monaco, March 18-21, 1979. Details from F. Cattabeni, G. Racagni and P. F. Spano, International Menarini Foundation — Piazza del Carmine, 4 — 20121 Milan (Italy). 7382 (M)

ROYAL SOCIETY
OF MEDICINE
1 Wimpole Street,
London W1M 8AE

The Section of Pathology of the Royal Society of Medicine has organised a two-day international Symposium entitled: QUANTITATIVE CYTOCHEMISTRY AND ITS APPLICATIONS for November 13 and 14, 1978.

Papers will be given under the following headings: Nucleic acid cytophotometry and Pathology; Haematology and Immunology; Toxicology and Pharmacology; Dermatology; Connective tissue disease; Maturation of the Graafian follicle; Cancer diagnosis and Research; and the Bioassay of Polypeptide Hormones.

Details of the programme and application forms may be obtained from the Sections Officer at 1 Wimpole Street (Telephone: 01-580 2070).

Closing date: November 1, 1978. 7330(M)

PERSONAL

RAPPORT is the discerning person's way of meeting new friends. Whatever your age, location or preferences, enrich your quality of life immeasurably with RAPPORT, the intelligent person's introduction service—and rediscover the joy of living. Details from RAPPORT, P.O. Box 94, Oxford. 7325(R)

LECTURES

The social and safety implications
of
Genetic engineering

A one-day public meeting organised by the Association of Scientific, Technical and Managerial Staffs, Friday, October 27, at The Pharmaceutical Society, 1 Lambeth High Street, London SE1.

The conference is open to all who wish to attend. Discussion will include not only the possible benefits and hazards of genetic engineering but also the question of reconciling public opinion and concern about safety, with the scientific and industrial progress in an increasingly technological world.

Speakers include: The Rt Hon Shirley Williams, Sir Gordon Wolstenholme, Clive Jenkins, Dr Sydney Brenner, Dr Jonathan King and Prof Bob Williamson.

For registration, which costs £4, please contact Donna Haber, ASTMS, Sutton House, 2-4 Homerton High Street, London E9 6JT or telephone 01-985 4792. Places are limited. 7374(K)

SCHOLARSHIPS

THE ROYAL SOCIETY
THE RUTHERFORD
SCHOLARSHIP

This award, for graduates of exceptional promise and ability who are under 26 years of age on May 1, 1979, is for experimental research in any branch of the natural sciences, with a preference for experimental physics if there are candidates of similar merit. It will be tenable for three years, from October 1, 1979 or such other date as may be arranged, in some part of the British Commonwealth other than that in which the applicant graduated. The value, adjusted to meet the circumstances, will be £2,250 per annum on appointment (under review) if held in the United Kingdom, with allowances for travel, university fees, etc. It will not normally be awarded to a graduate who has already held a senior research award.

Applications should be made as follows:

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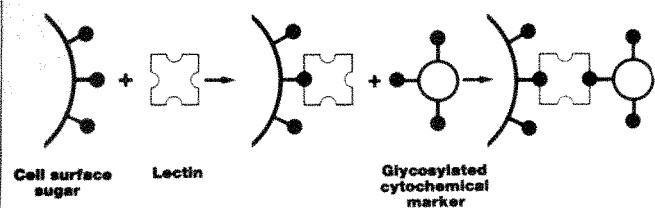
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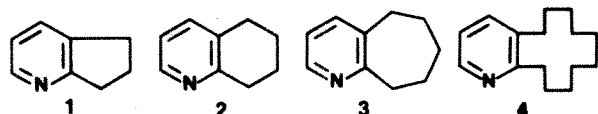
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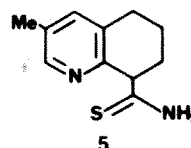


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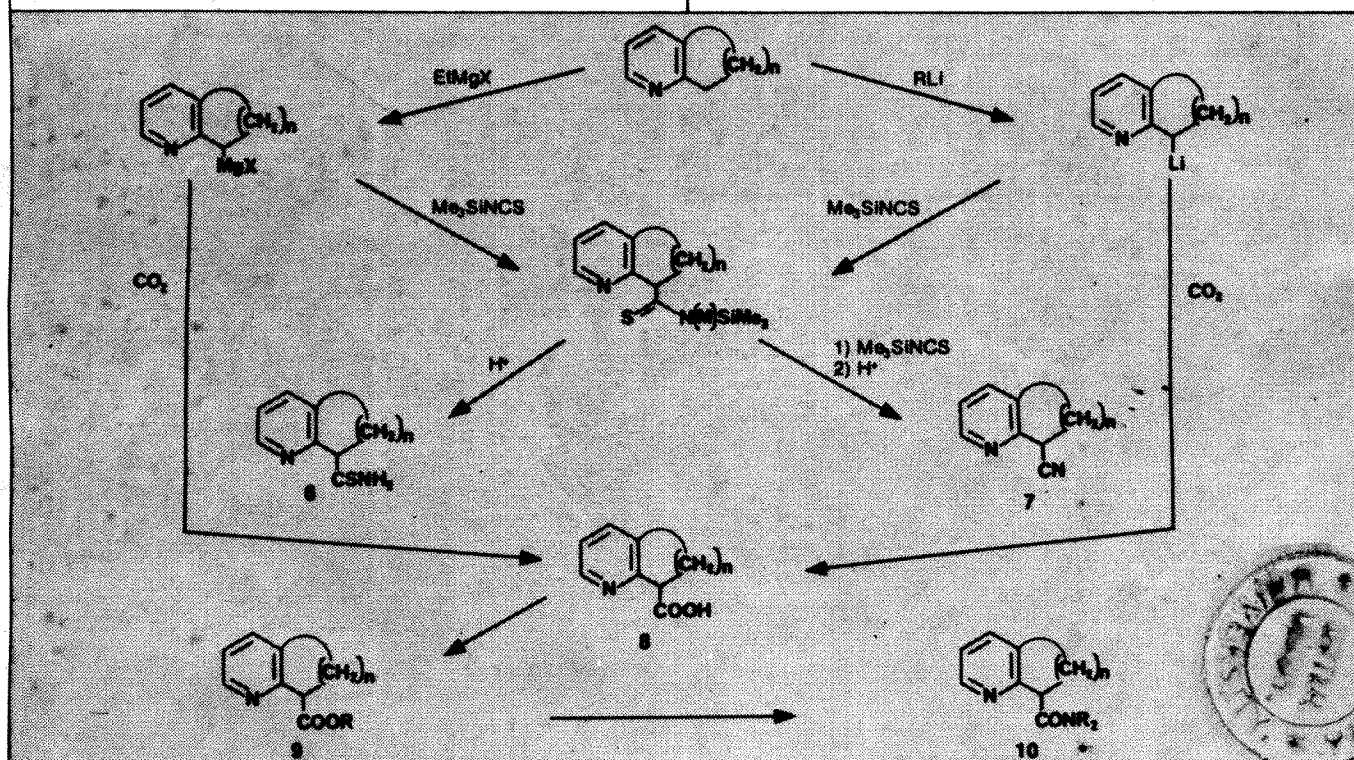
cycloheptenopyridine (3), and 2,3-cyclododecenopyridine (4).

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References:

For a review of the reactions of 2,3-cycloalkenopyridines, see H. Beschke, *Aldrichimica Acta*, 11, 13 (1978).

- 1) E.M. Kaiser, G.J. Bartling, W.R. Thomas, S.B. Nichols, and D.R. Nash, *J. Org. Chem.*, 38, 71 (1973).
- 2) R. Crossley, A.C.W. Curran, and D.G. Hill, *J. Chem. Soc., Perkin Trans. I*, 977 (1976).
- 3) A.C.W. Curran and R.G. Shepherd, *ibid.*, 983 (1976).
- 4) A.C.W. Curran and R.G. Shepherd, *Ger. Offen.* 2,459,631 (1975); *Chem. Abstr.*, 83, 131483w (1975).

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Cover picture

The Mojave Desert near Lucerne Valley, California. The horizontal distribution of the large green bushes (creosote bush) is regular and that of the small grey bushes (burro-bush) is contagious. See page 544.

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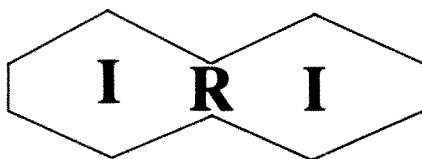
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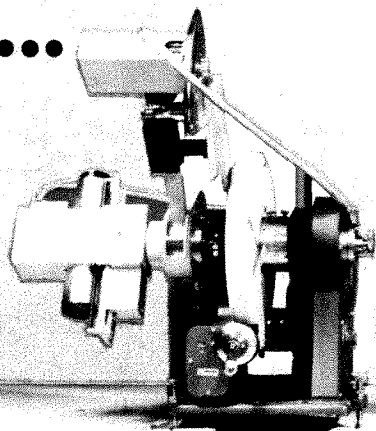
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12 October 1978

Linking science to technology: harder than it seems for Third World

THERE is a moral, a lesson and a set of danger signals in the three articles on science policy in Latin America in this week's *Nature* (pages 472-475). The moral is that linking a country's scientific activities to its technological needs can, in practice, be very much harder than the familiar political rhetoric would often have us believe. The lesson comes from the Andean Pact countries, who have been able to demonstrate how cooperation in research can help overcome some of the problems resulting from an over-dependence on scientific and technical skills of industrialised nations.

The danger signals relate to the increasingly important role of foreign investment—rapidly replacing the multinational company as the bogeyman of development—in determining scientific and technological research policies, not only in content but also in form.

Each of these observations must be seen in relation to a basic premise: that, in the words of a major study of the instruments of Latin American science and technology policy financed by Canada's International Development Research Centre, "the development of indigenous science and technology capabilities [is] an essential condition for achieving a certain degree of autonomy in decision-making on industrial development."

The experiences of both Venezuela and Peru in putting flesh on the bare bones of such statements have implications for other developing countries. Venezuela, for example, has attempted to develop a centralised strategy for its research policy through the creation of a National Council for Science and Technology (CONICIT), a model adopted by several other countries in the region. But the explicit goals of this strategy have been undermined by the consequences of an economic policy which, in pursuing rapid industrialisation, has encouraged joint ventures between foreign multinational companies and local capital. The result has frequently been to stimulate, rather than restrain, the flow of foreign technology into the country, providing companies with little incentive to support an indigenous research and development capacity.

Peru's problems have been slightly different. Since taking power in 1968, the country's military rulers have attempted to pursue a strongly independent economic policy; this has included not only the nationalisation of basic industries, but also the rapid increase in research budgets (up by a factor of 10 since the late 1960s) and support for both public and private research institutes. Subsequent moves to centralise science planning, however, seem destined to be moulded, at least in part, by the economic measures being demanded by the International Monetary Fund; these have been made the condition for bailing Peru out of the plight caused among other things by high military spending and the declining world price of copper.

In both Venezuela and Peru, science and technology policy is increasingly coming to reflect the domestic role played by foreign capital. And the danger of this is that it

skews decision-making to favour research and development policies, for example those concentrating on capital rather than labour-intensive production techniques, that benefit the upper and intermediate groups in society but have little impact on the plight of the poorest. Even where a "basic needs" technology is simultaneously advocated, this lies open to charges of "buying-off" the demands of the latter for an equitable share in the fruits of development.

Such issues can no longer be ignored by those concerned about the role of science in the development process. Fifteen years ago, the argument that the path to development lay in the rapid transfer of scientific and technological knowledge to the Third World, led participants in a United Nations conference to list priorities for such transfer in a "World Plan of Action". Today it is accepted that limitations and constraints on the transfer process are such that this philosophy is no longer adequate; indeed these limitations and constraints are the main focus of consideration.

This shift, embodied in the agenda for next year's United Nations Conference on Science and Technology for Development (UNCSTD), itself implies recognition that science and technology lie at the very heart of the development process. In practice, it is also coming to be recognised that policy matters in these areas can no longer be legitimately left to groups of experts, scientific or otherwise, but can only be forged through the type of bargaining and negotiation conventionally limited to more overtly political areas of national and international diplomacy.

Here there are lessons to be learnt from the recent UN conference on Technical Co-operation Among Developing Countries (TCDC) in Buenos Aires last month. Despite gloomy forebodings for the conference which predicted that the "real issues" would disappear in a storm of political acrimony, the meeting seems to have turned out relatively successful. In particular, the developing nations were able to unite behind a coherent and well-thought out position; and their combined strength was sufficient to add considerable bite to the draft plan of action which had been submitted by the UN secretariat, for example giving them greater control over the allocation of technical development funds.

TCDC marked an important shift from confrontation to negotiation that other UN conferences, including UNCSTD, would do well to try to follow. Certainly there are common scientific and technological interests between rich and poor nations; but given that these reflect broader social goals, no-one should be surprised that there are also conflicts. How to make such conflict productive, rather than merely self-defeating, is one of the most important issues that UNCSTD will have to confront. Encouraging joint research projects between developing countries, for example, can both provide practical benefits and increase the negotiating strength of the countries concerned; and ultimately it is this which determines who gets what share of the cake. □

DNA: let the public choose

Susan Wright, a historian of science at the University of Michigan, has followed the recombinant DNA debate closely. Here she comments on the recent public discussion of the revised NIH containment guidelines.

MUCH attention is currently being given to the concept of 'public participation' in policy decisions on technical and scientific matters. In referring to decisions on recombinant DNA research during a commencement address at the University of Michigan, Joseph Califano, Secretary of Health, Education, and Welfare, emphasised the importance of due process: "This means decisions made democratically through wide consultation, not by special elites". The tension in that statement, between "democratic" procedure and "consultation" (the results of which may still be digested by an elite group), points to a problem itself. Technical policy-making bodies are older than the growth of interest in participatory forms of decision making. They reflect an earlier faith in delegation of responsibility to high-ranking civil servants and expert advisors.

The political distance between current demands for participation in policy decisions on science and technology and the actual composition and procedures of policy-making bodies is nicely illustrated by the arrangements adopted by the NIH for decisions on recombinant DNA research. The elite nature of those arrangements was a principal subject of testimony at a hearing on proposed revisions to the NIH guidelines held at the Department of Health, Education, and Welfare in September before a committee chaired by Peter Libassi, HEW general counsel. NIH officials have claimed that all sectors have contributed to decisions on the guidelines through hearings and through the publication for comment of the guidelines, proposed revisions, and an environmental impact statement. However, testimony on the proposed revisions shows that many sectors see those procedures largely as a means for legitimising decisions taken within biomedical research circles rather than for facilitating participation in decisions.

The proposed revisions to the guidelines (10 August, page 411) are substantial: lowering of containment levels for many classes of experiment; exemptions which would take many experiments out of the guideline's control; and delegation of important responsibilities from NIH to local biohazards committees. Under the changes, local committees would be given wide responsibilities for recombinant DNA experiments. At a time when recombinant DNA technology promises to become as important for industry as it is for the universities, the revisions are a major step towards dismantling the central controls established two years ago.

All those who testified in support of the revisions were scientists and administrators with close ties to biomedical and agricultural research. Their reasons for approval were generally in line with those given by NIH director Fredrickson in his introduction to the guidelines. Delegation of authority to local biohazard committees was seen as a step in the right direction.

Critics of the proposed revisions included scientists and lawyers with ties to state and local government, trade unions, and public interest groups as well as to the universities. A concern expressed repeatedly at the hearing was that the circle of participation in decision-making has been largely restricted to a small group of scientists with close ties to NIH. According to Philip Bereano, a lawyer and professor in the University of Washington's Social Management of Technology Program, NIH decision making has

been dominated by an "old-boy network"—a reflection, ultimately, of the basic conflict of interest of the NIH in attempting to regulate an activity that it also promotes. Bereano's claim was supported by data from an NSF-funded study of the composition of institutional biohazard committees (IBCs) reported by Nancy Pfund of Stanford University. Preliminary results show that the proportions of workers, students, and members without ties to the institutions doing the research averaged less than 2% and that almost 70% of the membership was drawn from scientists and administrators in such fields as microbiology, biochemistry and molecular genetics.

Witnesses noted that growing industrial interest in recombinant DNA means that the NIH advisory committee will be called on increasingly in the future to make policy recommendations with far-reaching social implications. Fredrickson's proposal to "broaden [the committee] modestly as needed for expertise" and "to include, perhaps, a dissenter from NIH policies" was seen as an inadequate response to those demands. In written and oral testimony, representatives of the Attorney General of New York State, the American Federation of Labor and Congress of Industrial Organisations, the Coalition for Responsible Genetic Research, the Environmental Defense Fund, and Friends of the Earth called for substantial expansion of the committee to include members of trade unions, public interest groups, public health organisations, and government agencies with potential regulatory jurisdiction. In addition, Friends of the Earth lawyer Richard Hartzman stated that the proposed changes were so sweeping that they required a new environmental impact statement.

The proposal to delegate responsibility for oversight to local institutions also received strong criticism. Pointing to violations of the guidelines that have occurred at Harvard Medical School and the University of California, San Francisco, commentators suggested that to respond to those events by enhancing rather than by checking responsibilities of local committees was inappropriate. "NIH proposes a form of enforcement which institutionalises conflict of interest and provides even less accountability for the Director of NIH and the IBCs than the previous guidelines," wrote Marcia Cleveland and Louis Slesin of the Natural Resources Defense Council. This matter is also of concern to Assistant Secretary of Labor Eula Bingham and Senator Adlai Stevenson, chairman of the Senate subcommittee on Science, Technology, and Space, both of whom have proposed retention of more centralised controls.

Critics have also claimed that the closed form of decision-making had affected the quality of the technical evidence used in reaching decisions. "There has been heavy reliance on unrefereed, unexamined reports of small committees meeting in private which are then presented as the consensus of the scientific community," said Jonathan King, associate professor of biology at MIT. He stated that the results from those meetings had been used in an "extraordinarily selective manner in which no arguments that countered or failed to support claims were either quoted or referred to."

How will the DHEW handle testimony on the revisions? Will a full environmental impact statement be necessary? Libassi after the hearing declined to say what he would do, but, echoing Califano's words, he did say that he thought the issues raised were "not ones to be decided by scientists." As to his own role: "A lawyer's skills can be applied in technical areas as well as procedural ones." Clearly, several government sectors and the public will follow closely how the sentiments of Libassi and Califano are translated in terms of expansion of the present circle of recombinant DNA decision-making □

Where should LEP be built?

Europe's bid for a world lead in subnuclear physics in the 1990s brings CERN's future into question, writes **Robert Walgate**

EUROPEAN subnuclear physicists are brimming with enthusiasm for their next big project, LEP (see page 482), but building it will plunge CERN (the European organisation for nuclear research) into a hornet's nest of problems.

There is strong competition—tacit or not—between CERN and the West German laboratory DESY for the siting of LEP, a large electron-positron storage ring; and there is controversy over CERN costs, with large tax-free wages accounting for over half CERN's expenditure. The French, German, and British governments are insisting that the CERN budget be brought down from its present 600 million Swiss Francs (MSF) a year to 560 MSF by 1981, but LEP can only be built at reasonable speed (in under a decade) if CERN has some 600 MSF a year.

Meanwhile, the tacit international understanding that the next machines would be continental, with the USSR building a 3 TeV proton synchrotron, Europe building a 100 GeV electron-positron storage ring (LEP), and the US building Isabelle (a 400 GeV proton storage ring), has been violated with the news that the US is also looking at a LEP-like project. Burton Richter, the Nobel laureate who led the discovery of charm with LEP's progenitor, SPEAR, is reputed to be pressing the US government for an American LEP. And as the US Department of Energy is discussing collaboration with Japan on a number of research issues, including subnuclear physics, it is conceivable that Professor Richter will find Japanese money to build a LEP ahead of Europe despite US expenditure on Isabelle and Fermilab. The race is on, and as one CERN physicist put it "the first to the money will get LEP".

The future of LEP in Europe is confused by a number of factors, one of which is the rising eminence of the German laboratory DESY. "Four years ago we were nowhere", a DESY physicist told *Nature*. "Now we are on top, and physicists are clamouring to work here". The root cause is the realisation that electrons, particularly in collision with positrons, are the cleanest probes of the nature of matter on small scales. DESY is Europe's largest electron laboratory. Further, DESY has succeeded in building PETRA.

PETRA is the world's largest storage ring for electrons and positrons, with (at 19 GeV) twice the energy per beam of SPEAR (and its equivalent at DESY, DORIS). It will begin experiments on

22 October, eight months ahead of schedule, though it may take a month or two before its intensity reaches design levels. It will probe new quark combinations (like 'bottomonium') and most likely reveal new quarks ('top' is expected) and leptons. And with an experiment devised by Samuel Ting (co-winner with Burton Richter of the Nobel Prize for the discovery of the 'hidden charm' particle J/psi) it will test the unification of the weak and electromagnetic forces proposed by Abdus Salam and Steven Weinberg. PETRA will give European subnuclear physics a world lead for five years or more.

Experience in building and running PETRA also gives DESY physicists a European lead in the design considerations for LEP. But there was not a single DESY representative on the 63-man CERN study of LEP, recently completed (CERN/ISR-LEP/78-17, the 'Blue book'). Professor Schopper, the director of DESY, argues that the best contribution that DESY could have made was to build PETRA, rather than to work on "a paper study"; and anyway the CERN team spent a long time at DESY, discussing the PETRA design. Schopper points out that several of the design points in the CERN study are a simple scaling-up of PETRA elements—the rf cavities, for example, and the vacuum tube. DESY physicists were of course spending 16 hours a day building PETRA, but the DESY absence from the study has also been interpreted as a political manoeuvre; so much so that the brief presence of several senior representatives of DESY, including the builder of PETRA, Gustav Voss, at the CERN summer study on LEP recently was seen as something of a thaw.

All this would matter little if it did not involve the future of CERN. The CERN budget is under pressure from its staff (some 4,000 people, mostly technicians) whose promotions have fallen to a low ebb, and who are, of course, keen to maintain salaries at their high level. (50% of the staff live in France, where they could earn only half the sums they cull from CERN; a study, called RESCO, is underway on the social conditions and remuneration of workers at CERN.) The budget is also under pressure from the Swiss Franc, in which CERN subscriptions and salaries are payable. The franc has strengthened from 12 SF to the £ in 1966 to 3 SF at present, quadrupling the British subscription over that



Who should CERN fear most: Chancellor Schmidt (left) or DESY director Herwig Schopper?

period. And the budget is under pressure from the other direction from the member states, who want to keep their expenditure on subnuclear physics in proportion to their other research costs. The CERN-built LEP would cost some 1000 MSF, but as a French physicist commented, "what will that mean in 10 years in pounds, or French francs, or even Deutsche marks?"

According to one influential physicist, the European Committee for Future Accelerators (ECFA), which has been charged by CERN members with producing a firm proposal for LEP, has in its hands a very delicate decision. The committee, he said, has to consider which course of action for LEP would preserve the best future for subnuclear physics in Europe. LEP at CERN's Geneva site might price itself out of the market. And CERN without LEP in the 1990s would be a dying laboratory. The question seems to be whether the focus for subnuclear physics in Europe should move for the 1990s from Geneva to Germany (though there are other contenders, notably France; only Italy has ruled itself out).

CERN defends itself strongly, pointing out that a new laboratory would need a new infrastructure, entailing extra cost; that LEP at Geneva would allow collisions between the electrons of LEP and the protons of the SPS, allowing an ultradeep probe of proton—and perhaps quark—structure; and

that the LEP tunnel, once built, could be used for many other purposes at CERN, with its complex of accelerators

Over at DESY, Professor Schopper is at pains to protest that there is no competition between his laboratory and CERN; but other DESY physicists see it differently. "We are planning moves like a chess game", said one. And DESY can afford to sit tight for the moment: it is in a strong position, technically and politically. On the technical side, pressure from physicists for the highest possible energy favours DESY. High energy means large ring size to reduce the synchrotron radiation losses from the circulating electrons (and so keep down the power bill). But the CERN site is restricted by the Jura mountains to the north. DESY's presently favoured site, in a national park 40 km south of DESY near Hamburg, is not so restricted.

However, an early CERN study of a 100 GeV machine (LEP-100) was abandoned. The 'Blue book' argues that there will be many difficulties in handling electron beams at 100 GeV. The summer study concluded that "improving the energy [beyond 70 GeV] requires far more knowledge of a theoretical and technical nature before a realistic design may be settled upon"; but Schopper says that such conclusions are premature, and based on a too-pessimistic interpretation of work on the problem at DESY. In principle DESY should know, but it is not easy to disentangle physics from politics on this point.

The present half-cost LEP planned by CERN is a project for 70 GeV beams (LEP-70), with a possibility to upgrade to 100 GeV if superconducting rf power cavities become feasible. One of the key tasks for LEP will be to produce the intermediate vector bosons, which have masses and properties such that the neutral Z^0 could be produced with single beam energies of about 45 GeV, while the charged W^+ and W^- would need 80 GeV. The Higgs bosons, which are essential to the unification of the forces, and hold the key to the masses of the quarks and leptons, require the highest possible energy. LEP-70 is not therefore the most comfortable machine for physicists—100 GeV would be better—although there will be a cornucopia of treasures on LEP-70 from the "resonance" in the interaction at the Z^0 , which should yield a thousand-fold increase in interaction rate.

ECFA is just beginning studies of the machine parameters for LEP, under the chairmanship of Professor Antonino Zichichi—who intends to be entirely "Galilean" in his considerations. In other words he wants his committee to come up with the physi-

cists' perfect proposal, without regard to politics. That study is due at the end of 1979 for presentation to ECFA and to CERN Council. It will be interesting to see whether it more nearly approaches LEP-70 or LEP-100. ECFA will then have to juggle with site and politics to come to its recommendation.

John Adams, CERN's machine-building director-general, said in Geneva recently that he would like physicists to reach agreement on a site "by the end of next year". He added "If the physicists can't decide where to put the thing they can't blame governments for delays". And there are further battles to be won at government level.

Germany lost the fusion project, JET, to the UK, and might welcome a new international laboratory. Professor Schopper insists that any extension of

Argentina— where thought is a crime

DR CLAUDIO BERMAN, a professor of psychiatry held incommunicado in Cordoba prison in Argentina without specific charges since April 1976, was released last month, following pressure from the US National Academy of Sciences' Committee on Human Rights, the Israeli embassy in Buenos Aires, and international human rights groups.

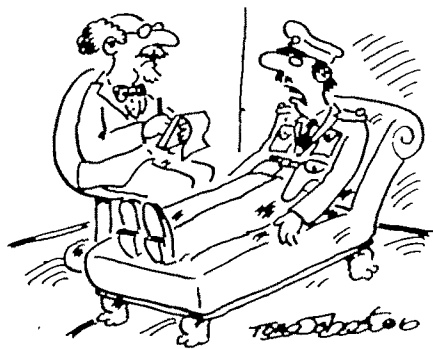
Dr Bermann was released into the custody of an Israeli representative only when he was actually aboard the aircraft bound for Israel. On arrival in Israel, he avoided any formal contact with the Press, apparently fearing for the safety of fellow-prisoners still in custody. It was, however, possible to learn informally some interesting sidelights on the position of psychiatrists in Argentina, and, in particular, the reason why so many psychiatrists figure in the lists of political prisoners and 'disappeared persons'.

"Thought is a crime there," explained a friend of Dr Bermann's cynically. More particularly, psychiatry which, as a therapeutic discipline, aims at establishing the autonomy of the individual, is seen by the present regime as potentially subversive—an opinion which draws some substantiation from the fact that, until 1973, the Argentinian Federation of Psychologists did, as a matter of fact, have a large number of members whose political leanings were leftist or liberal. Freudian psychology is banned from the universities (where it is seen as a Marxist subversion unlike the Soviet Union where it is condemned for being anti-Marxist!), and, according to

DESY would be "fully international", with contributions from no country ruled out (including Japan). And the small countries at CERN will not be happy with the closure of CERN's low energy synchrocyclotron and the intersecting storage rings, as will be necessary if CERN is to build LEP within its 600 MSF ceiling. The Scandinavian countries, in particular, make good use of the synchrocyclotron for nuclear physics, and the intersecting storage rings are now ready for nuclear physics experiments with light nuclei.

CERN's problems with LEP are manifold, and it is no wonder that before John Adams gave his recent talk at CERN on "steering LEP" to an enthusiastic audience of physicists his colleagues told him, "And for God's sake be cheerful"

Robert Walgate



"I keep thinking I'm two psychiatrists"

another expatriate psychologist, university teaching in psychology goes no further than the writings of St Augustine and St Thomas Aquinas.

Furthermore, said the same psychologist, difficulties can arise in the therapeutic situation. A psychiatrist might attempt to treat a patient's anxieties by presenting the current regime as an unpleasant, unavoidable fact of life, which one must somehow learn to live with. Further there are reports of members of guerilla groups actually receiving treatment from psychiatrists without the psychiatrist actually knowing anything about this side of their activities—a proposition which seems at first glance somewhat unlikely, but which, within a group therapy context, is quite feasible.

Acting on suspicion of some such link between psychiatrists and guerillas, the Argentinian authorities have, according to Dr Bermann and his friends, been putting pressure on practising psychiatrists to betray their professional confidentiality, and, in some cases, it would appear that agent-

provocateurs have been used, posing as guerrillas in need of treatment, to determine the "loyalty" of the psychiatrists.

According to Dr Bermann, when first arrested, he was himself tortured in an attempt to establish a link between him and the guerrillas. When the authorities finally realised that he had no information to give them, he was put in a cell with other "political prisoners who knew nothing," from which the sounds of others being tortured could be heard.

Dr Bermann, as a professor and former director of a psychiatric clinic, ranked as an important prisoner—and as such was chosen as a kind of hostage when President General Jorge H. Videla visited Cordoba last year. Twenty such hostages were arrested—though as incommunicado prisoners they could do nothing to avert any possible attack on the presidential party. Nevertheless, their fate depended on the safety of the President and his entourage, according to an equation

which seems to be a peculiarly Argentinian way of assessing the value of its leading citizens.

If there was any attempt on the President's life, they were told, all 20 would be shot; if a General was killed, 14 would be shot, if a colonel, eight, and if any other official or policeman were killed, only two. So, by the new Argentinian mathematics, it would appear that one leading psychiatrist equals half a policeman!

Vera Rich

US producers fear ban on 2,4,5-trichlorophenol

SOME of the producers of 2,4,5-trichlorophenol fear that it might be banned in the US. The Environmental Protection Agency (EPA) has recently issued a notice of 'rebuttal presumption' against current and continued registration of trichlorophenol and its salts. The notice—the first of several which the agency intends to issue—says that they exceed "certain risk criteria".

The issuing of a rebuttal presumption is not, says the agency, notice of intent to ban the products. Such action would only be taken after careful evaluation of all the evidence had shown that their continued use would cause "unreasonable adverse effects to the environment". However, the notice does require that the users and manufacturers of trichlorophenol submit evidence to the EPA, proving that its continued use does not constitute a hazard.

This is not the first time that the EPA has reviewed these particular chemicals. 2,4,5-trichlorophenol is used in the manufacture of the herbicide 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) and of the bactericide, hexachlorophene. The reaction process used to make trichlorophenol produces an extremely toxic by-product, tetrachlorodibenzo-p-dioxin (TCDD). It is the presence of TCDD—a known teratogen and potential carcinogen—which is causing the current concern.

2,4,5-T has been reviewed recently by government committees in the UK and Australia, both review bodies pronounced the herbicide safe if used for the purposes for which it is intended. Manufacturers are less optimistic about its chances in the US.

Among the most recent evidence that the EPA will examine are two sets of documents relating to the safety of the manufacturing process. The first is the Italian Parliamentary Commission's

report of its investigation into the accident which occurred in the ICMESA trichlorophenol reactor near the Italian town of Séveso nearly two years ago. The second is the report of ICMESA's Swiss owners, Givaudan and Hoffman-La Roche, on their own research into the cause of the accident.

Their report is in reply to the commission, but it has been prepared especially for the EPA. The commission's report, which runs to several hundred pages, accuses Roche and Givaudan of being less than candid about their operations at ICMESA and of running an unsafe chemical plant. The commission also claims that ICMESA was producing trichlorophenol without an up-to-date 'fire precautions certificate'; that it failed to inform the Labour Inspectorate that it had begun production of trichlorophenol, that it failed to notify the authorities of the accident for twenty-seven hours; and that when it did inform them, no mention was made of the release of TCDD.

ICMESA produced 130 tons of trichlorophenol in 1975 and 1976; a sizeable amount, according to the Italian commission. Roche and Givaudan will contend in their report that compared to the output of companies in the US and Germany this is a small amount. The commission's suggestion that the trichlorophenol reactor was poorly designed, is refuted by Givaudan, who claim that their design incorporated all the features necessary at the time for safe reactor operation.

The ICMESA reactor did not incorporate a dump tank to collect unwanted discharges from the reactor vessel. Givaudan thought this unnecessary, in view of the plant's other safety features. The commission, however, thinks this was a serious error,

A safety disc on the vessel was designed to rupture at the pre-set pressure of 3.5 atmospheres, and so discharge TCDD over Séveso. The Italian report states that a lower value would have caused a rupture at a less advanced stage of the reaction, with less disastrous consequences. Givaudan argue that the disc was incorporated to cope with mishaps at the beginning of the trichlorophenol reaction process—the accident at Séveso happened some six hours after the reaction was completed—and that the rupture pressure of the disc was selected for sound chemical reasons.

Central to the concern about the health of Séveso's inhabitants is the two week delay in their evacuation from TCDD contaminated areas. The commission says that Roche and Givaudan did not act swiftly enough to determine the extent of contamination. The companies contend that after a map of the polluted area had been made—a task which took two weeks—the proper authorities were informed and recommended by Roche to evacuate the area. Roche says that the authorities resisted initially until they appreciated the gravity of the situation.

Many scientists think the Séveso residents were exposed to low levels of TCDD. The commission disagrees, and recommends further studies to assess the long-term effects to health. TCDD is a potential carcinogen, and numerous scientists consider such studies essential. The International Agency for Research on Cancer (IARC), which has recently published a new report on the polychlorinated dibenzodioxins is currently assessing the chemicals' carcinogenicity. It is reviewing evidence from a number of the fourteen accidents which have occurred in trichlorophenol plants since 1949.

Alastair Hay

Venezuela: still out in the cold

If Latin American research centres were graded by location, the greatest number of stars would surely be awarded to the Venezuelan Institute for Scientific Investigation (IVIC). Set on a mountain-top retreat 20 miles outside Caracas, its lush forest surroundings complemented by imposing works of modern sculpture, IVIC has all the environmental requirements of the archetypal ivory tower.

Yet its very isolation reflects a major complaint of Venezuelan scientists. Despite concerted efforts over a period of years to link scientific research to the country's technological needs, their activities remain marginal to the country's main economic and industrial activities.

IVIC itself, through a combination of generous private and public funding, has become, since its foundation in 1959, one of the leading basic research centres in Latin America. Its facilities in fields such as biophysics and analytical chemistry attract scientists from all over the continent. They often rival those in the laboratories of industrialised countries in which many of IVIC's research workers received their initial training.

The institute has also been actively developing its own postgraduate courses. There is a proposal before the next meeting of the United Nations Educational, Scientific and Cultural Organisation (UNESCO) in Paris, for example, that IVIC should become an information centre for postgraduate studies for the whole Latin American region.

Despite its scientific successes, however, the institute and its activities remain highly vulnerable to the effects of the country's changing fortunes, according to its director, Dr Luis M.

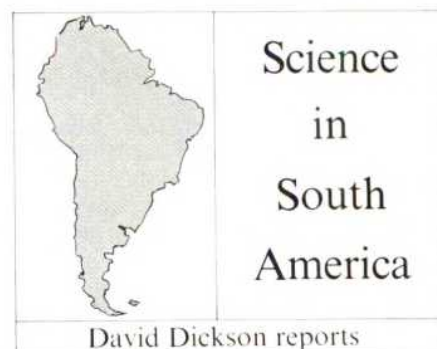
Carbonell. Earlier this year, for example, a 7% across-the-board cut in public expenditure—a response to a growing balance of payments problem and escalating inflation—resulted among other things in a six-month moratorium on travel to international conferences.

IVIC's vulnerability is itself a reflection of the extent to which research in Venezuela remains marginal to the country's main technological activities. A policy of pursuing rapid industrialisation while oil revenues continue to come in has largely been carried out through the transfer of technology from abroad; and although research and development expenditure, judged on a per capita basis, is among the highest in Latin America, it is still only 0.28% of the gross national product, less than a tenth of the proportion spent by many of the industrialised nations.

An attempt to rationalise the country's scientific efforts and link them more directly with its technological needs was made ten years ago with the setting up as an offspring of IVIC the National Council for Scientific and Technological Investigation (CONICIT), one of a number of similar planning institutions which have been established in various countries in Latin America.

"The marginality of science in Venezuela has largely been the result of a lack of communication between the scientific and the productive systems. Since its creation, CONICIT has been taking action to confront this situation," according to the council's president Dr Pedro Obregon, a widely-respected plant geneticist.

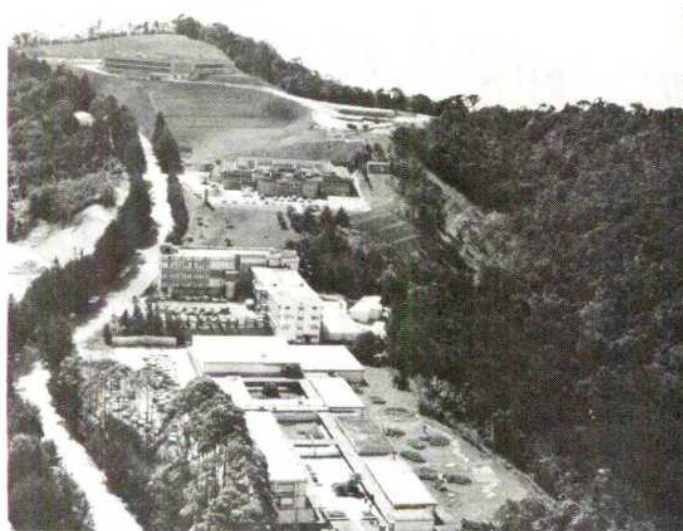
One area in which the council has enjoyed considerable success has been



in increasing the skills of those responsible for purchasing foreign technology in choosing that which is most appropriate to the country's needs; centres have already been established in London and Vienna to assist in this purpose and another will shortly be opened in Washington.

CONICIT has also been successful in stimulating new research initiatives in fields such as health and agriculture. In the latter case, for example, it recently opened a Centre for Agricultural Technology. Yet despite concerted attempts, CONICIT has so far had little success in generating the interests from the direction that, at least as far as the country's economy is concerned, matters most, namely sectors such as the chemical and steel industry.

Such industries, often under the government's rapid industrialisation policy and its support of joint ventures between national and foreign capital, have preferred to import technologies from abroad, rather than building up an indigenous research and development infrastructure. "The problem has been getting local industry interested in local technology" as one observer puts it; and the result is that CONICIT has been relatively unsuccessful in achieving one of its prime goals, namely increasing the country's technological



Institute for Scientific Investigation: five-star location but vulnerable to budget cuts and inflation, says director Luis Carbonell (above)

independence from foreign interests.

"Apart from opening the minds of many people to the problem of relating science to technical needs, and providing funds for training, I don't think CONICIT has been able to do much more in achieving its original goals," says Dr Carbonell of, IVIC who points out that 95% of patents taken out in Venezuela are still foreign, and that of the remaining nationally-owned patents, hardly any have been exploited.

CONICIT's problems are reflected at IVIC itself. One of the effects of the marginalisation of science is, it is claimed, a lack of respect for basic science as a profession; salaries are considerably lower than in universities, where teaching loads are so heavy that relatively little research is carried out.

In recent years IVIC has, for both ideological and financial reasons, been attempting to shift its focus away from a primary concern for basic science to embrace more applied subjects as well. The institute now has a small but active programme in solar energy research, concentrating in particular on methods of growing cadmium crystals, and is planning an investigation into techniques for the gasification of coal.

The institute also has a small research reactor, used not only for train-

ing engineers in handling nuclear materials but also for producing isotopes for medical and industrial use. At present, Venezuela has no plans for a nuclear energy programme; "but we are training people to make the right decisions, when these become necessary," says Dr Carbonell.

So far, however, as with CONICIT, industry's response to IVIC's efforts in the field of applied science has been disappointing. "Although lip service is given to the need to develop applied research, there does not really seem to be much real demand for it," according to Dr Marcel Roche, IVIC's first director and now editor of the Latin American journal *Interciencia*.

"Venezuelan industry tends to go for the maximum benefits with the lowest risks. The result is that research remains peripheral to the interests of industry; and this is a situation which has not been changed by CONICIT."

One attempt to improve things has been the drafting of a new law, similar to that which was introduced in Peru in 1971 (see below), which would oblige all industrial enterprises, to contribute a proportion of their gross profits to a research and development fund administered by CONICIT.

"In the future, it is essential that

research receives financial backing from the industrial sector. Not only will this provide research institutions with more resources, but it will also help protect industry from foreign interests," says Dr Obregon.

If the proposed law is accepted by the Venezuelan Congress—and this will depend largely on the outcome of the forthcoming presidential elections, as well as new Parliamentary elections next year—the result will be to put CONICIT in a much stronger position not merely to plan, but also to carry out a coordinated research strategy. It could also increase its budget, currently less than \$2m a year, by a factor of up to ten.

And what about the future of basic science? Dr Roche, a firm supporter of the need for developing countries to build a basic research capability to encourage technological independence, is optimistic.

"In my lifetime I have had the opportunity to see Venezuelan science grow at a relatively impressive rate. When I began to do research, there were in the country only six or seven professional investigators; now there are hundreds, well prepared and competent." □

Peru rings the changes in science policy

PERU has become the setting for a fierce debate on the respective roles that scientists and government planners should play in the design and administration of a national science policy. The focus of the debate is a proposal now before Peru's military leaders to upgrade the country's National Research Council, at present a primarily advisory body made up of eminent scientists, and to use it as the basis for establishing a "national system for science and technology"

The proposal is being resisted by groups such as the Institute for the Research in Industrial Technology (ITINTEC), which claim that a strongly centralised research policy would restrict the advantages of flexibility gained from the present system of relatively autonomous research institutes, each linked to separate government departments.

Others, including members of Peru's National Planning Institute—a government agency whose director has ministerial status—while agreeing on the need for a central programme for science and technology, argue that it should be conceived within, rather than parallel to, the existing social and economic planning machinery.

The root of the current conflict can be traced back to an ambiguity that

has existed since Peru's military government came to power in 1968. Keen to restrict the power of foreign interests and promote the country's technological independence, the government has encouraged the growth of a network of sectoral research institutes. There are now about 230 separate research institutes in Peru, half in the private and half in the public sector. And many, such as the Instituto del Mar del Peru (IMARPE) in Callao, enjoy an international reputation. At the same time, however, the government created as one of its first post-revolutionary acts the National Research Council, which theoretically has responsibility for establishing a national science and research policy.

"The problem is that when the government set up the council, it did not provide it with the authority or the resources to carry out the responsibilities it had been given," according to the council's current president, Dr Antonia Pinilla. "In consequence, the council has not been able to play the leadership role that it should have done."

As a remedy, Dr Pinilla, a previous Minister of Labour and a co-founder of the private University of Lima, has proposed a "national system" for coordinating the country's research

efforts (Such a system has already been promised by the government in its Tupac Amaru Plan for returning to civilian rule by 1980)

The system would have two main components. The first would be a National Council for Science and Technology, a "normative body" responsible for setting policy goals and research priorities. The council would be made up of eminent scientists and those responsible for the various scientific and technological research institutes. The second would be an administrative body known as the National Institute for Science and Technology. In addition to carrying out the policies determined by the council, the institute would be responsible for setting up both an Advanced Centre for Science and Technology—a type of "Peruvian MIT", as Dr Pinilla describes it, concerned with postgraduate studies and research—and a national Centre for Scientific and Technological Documentation.

Dr Pinilla has suggested that the head of the national system act as both president of the council and as director of the institute, and that in recognition of the central role of science and technology in the country's economy, he or she be given equivalent ministerial status to the director of the

National Planning Institute.

The proposed science policy machinery has received enthusiastic support from amongst others, the science policy secretariat of the United Nations Educational, Scientific and Cultural Organisation (UNESCO), which has been promoting the "minister of science" approach for a number of years.

Others inside the country, however, are less enthusiastic. Despite Dr Pinilla's promise that the system will "respect the academic autonomy" of individual research institutions, they are worried that attempts will be made to impose unrealistic goals and objectives while limiting flexibility.

Particularly concerned are members of ITINTEC, an agency set up by the government in 1971 to stimulate research in the various areas of productive industry. At present, although working to support areas that have been given priority by the government, ITINTEC is able to operate relatively independently because its main income comes directly from industry.

Under the law establishing ITINTEC, all companies in Peru have to set aside 2% of their gross profits each year for research. A company can either submit for ITINTEC's approval a research project which it wishes to carry out itself, or hand the money over directly to ITINTEC, which will use it to finance its own research.

The model seems to have worked quite well, and the Venezuelan government is contemplating introducing a similar system (see above). Over a third of ITINTEC's research projects are carried out by universities, and the institute has established centres for wood technology and non-metallic materials, as well as what is claimed to be the best technical information service in Peru.

Furthermore ITINTEC has recently received a \$2 million grant from the US Agency for International Development to investigate the application of "appropriate technology" ideas to Peru—a project which will include studying the possibility of restoring the famous agricultural terraces built by the Incas, now realised to be a highly efficient means of using soil and rain-fall in mountain areas.

Under the proposed new system, although ITINTEC and other research institutes would remain responsible for developing and carrying out their own research programmes, they would be required to present plans and budgets to the National Council for prior approval. "We feel strongly that technological research and development should be carried out within each sector of industry, without being subject to any strong centralised control, which we have been lucky enough to avoid in the past," according to Dr



Inca terracing: old but efficient

Jorge Boggio, ITINTEC's director of technology. "Also, although we agree on the need to coordinate the country's scientific and technological research activities, I think it is wrong to give responsibility to a body whose members have had little experience of technological development.

Related views are expressed at the National Planning Institute, which has had a strong influence on carving out Peru's highly nationalistic industrial policy in recent years. "I feel that our country needs a very clear and long-term plan for science and technology, but it must be built on the long-term objectives of socio-economic planning," says the director of the institute's office of research and planning, Dr Fernando Gonzales Vigil. "We need to determine broad priorities first, and then inside these priorities to put an emphasis on specific areas of knowledge, rather than the other way round."

Much will depend on the attitude of the new Minister for Industry, Admiral Jorge Du Bois Gervase, a former head of the industrial service of Peru's navy and therefore, it is claimed, well aware of the problems of relating research to operational needs.

Another factor will be the effects of the austerity measures that the government has been forced to take to avoid defaulting on its loans from the International Monetary Fund. Although a proposal to eliminate industry's required contribution to ITINTEC has been successfully headed off, for example, the institute remains vulnerable.

But whatever the outcome, many feel that the policy finally adopted is likely to be one that increases support for the private sector, and weakens the role of state planners. "This would be the more logical outcome, taking into account the various national and foreign interests," says Dr Vigil. □

Andean Pact propose research tax on 1st world

ALREADY bidding for a place on the agenda of next year's UNCSTD meeting in Vienna is a proposal that has been put forward jointly by a group of Latin American countries and the Economic Commission for Latin America (ECLA) to establish a new fund for supporting technological development ventures in the Third World.

The countries concerned—Bolivia, Colombia, Ecuador, Peru and Venezuela—are members of the Andean Pact, an economic federation formed in 1969 to harmonise and coordinate the economic and technological policies of its constituent states.

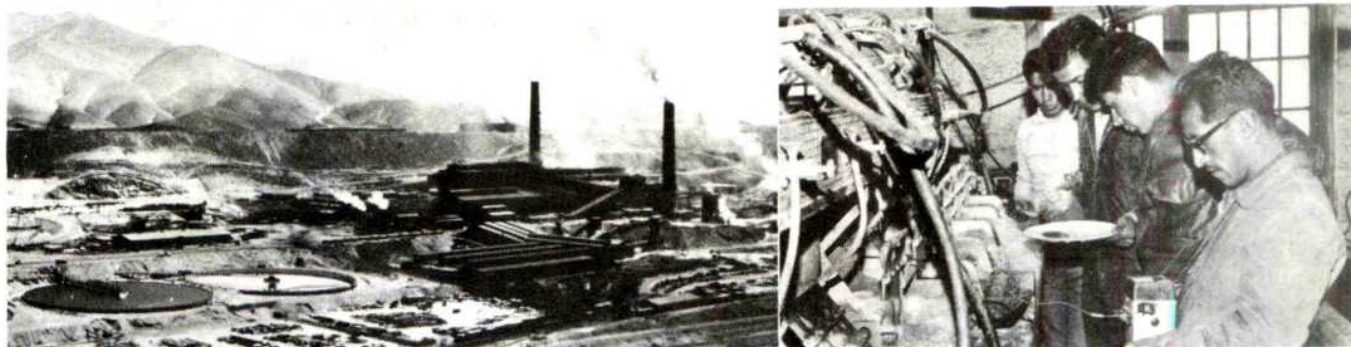
From the beginning, the Andean Pact countries have expressed, mainly through the reports and studies of its secretariat's technical group, the Junta del Acuerdo de Cartagena, fierce criticism of the way that existing technology transfer and patent licensing policies have increased the dependency of the developing on the developed world.

The proposed fund is intended to strengthen the indigenous research and development capacities of developing countries. Contributions would be based on the size of the balance of trade on manufactured goods between rich and poor countries, with the general assumption that the greater the imbalance, the greater will be the technological gap.

Details of the proposals are now being revised in the light of comments made when the idea was discussed at the UNCSTD regional meeting for Latin America in Panama in August. Although doubts were raised by some of the countries present—mostly those claiming to be at an "intermediate" stage of development—others gave it enthusiastic support.

The Pact's position on technology, spelt out in a joint Decision 84 signed in June 1974, is based on the premise that if a country is to have control of its own development programmes, it is not enough merely to control the investment of capital (for example by regulating the financial activities of multinational companies). Equally important is control over the development and use of technology.

"It is easy to show, for example by looking at the way that the patent system has been used, that unless you have people who can control the



Cooperation to improve production technique. Left: coppermine in Chile. Right: ore analysis in Bolivia.

technology—and not just capital alone—you will remain in a position of dependence on the industrialised nations,” says Dr Luis Soto-Krebs, a Chilean metallurgist who is director of the Junta’s science and technology policy group.

One paragraph of Decision 84 states that “the high fixed costs of research and development necessitate careful choice of research priorities and the avoidance of duplication of effort”. The member countries agreed to develop sub-regional methods for generating new technologies through the application of local knowledge and research, and also to apply these techniques to some pilot projects.

One of the first projects was to improve techniques for copper production, a major source of revenue for Peru, Bolivia, and Chile (which was until 1975 a member of the Andean Pact).

Two problems were tackled simultaneously. The first was to develop new hydrometallurgical techniques for extracting copper from low grade ores; the second was to build up, virtually from scratch in the case of Bolivia, indigenous research and development that would cut down dependence on foreign technology and know-how.

Engineers and scientists from Peru and Bolivia went to Chile—the most advanced of the three in copper technology—for a one-month seminar in 1974 on the theory and laboratory-practice of copper extraction. They also spent some time working in different types of extraction plants in Chile. “The basic idea was that everyone in the project should have a common language; that people in the lab should know about the problems of the plant, and vice versa,” said Dr Soto-Krebs.

Members of the Bolivian team, which concentrated on methods of using conventional sulphuric acid leaching of the oxide ores, set to work on the design of a plant planned for the Corocora area by the Bolivian Mining Corporation (COMIBOL) that would process between 300 and 600 tons of ore a day.

Much of the work involved basic geological studies of the site of the

plant, and of the properties of the ores—often using testing facilities made available by Peru. Three years’ work resulted in detailed plans for what is proudly called “the first Bolivian-designed copper extraction plant” being handed over at the Junta’s headquarters in Lima earlier this year.

Officials hope that capital for building the plant will soon be made available, probably by West Germany, which helped to subsidise the initial research and development work. They also point out that the team which drew up the plans now has the experience to tackle other similar projects.

The Peruvians carried out much of their project in a research laboratory set up in La Oroya, the centre of Peru’s mining industry. The aim was to investigate ways of exploiting bacteriological leaching techniques for concentrating the ore, already known about by larger copper companies in the US, but little used or understood.

With the help of a German scientist who had been studying the relationship between bacteria and crystal structures, and two Canadian scientists who had been looking at applications of bacteriological leaching, Peruvian biochemists were able to separate out and reproduce those bacteria most suitable to local ores. Starting from ore samples no larger than 300 grams, the research built up until it was able to build and operate a pilot plant. “It soon became clear that we were almost in the front line of this research, and we came out with a method for very fast leaching very different from what was already being done,” says Dr Soto-Krebs.

The pilot plant is now producing two tons of copper a day at a considerably lower cost than conventional extraction techniques. Bacterial leaching also has the advantage of being non-polluting—since there are no exhaust gases to discharge into the air—and of being applicable to much lower grade ores than could previously be handled.

Since the success of the two copper projects, two other technological development projects have been set up by the Junta along similar cooperative

lines, one studying the use of forest products, a second looking at nutrition and food production. Other projects in the pipeline include a proposal for a joint investigation of techniques for the gasification of coal, of which Venezuela and Colombia in particular have large deposits.

“Too often our people are convinced they can do nothing . . . but we have now convinced people at the highest level that carrying out our own technological development is not a dream, and that investing in the generation of such technology is not useless,” says Dr Soto-Krebs.

Financing for similar types of projects could, it is claimed, be provided by the new fund which is being suggested to UNCSTD. The idea for such a fund was first raised at a meeting in Caracas earlier this year by Dr Francisco Sagasti, the Peruvian co-ordinator of a five-year study of science and technology policy in Latin America that has been financed by Canada’s IRDC.

“One area of top priority in stimulating cooperative research ventures between developing countries is providing financial support,” says Dr Sagasti. “What is being suggested is that the developed countries make available a sum proportional to the imbalance in trade in manufactured goods with the developing nations, calculated on a regional basis.”

Dr Sagasti estimates that, using 1977 trade figures and calculating on the basis of 0.25 per cent of the trade imbalance in manufactured goods, Latin American countries would receive about 60 million from the USA, Japan and members of the EEC alone.

“Such a mechanism would help to increase significantly the flow of funds for scientific and technological activities in the Third World, thus initiating a gradual process of redistribution of the world science and technological effort,” said Dr Sagasti. □

NEXT WEEK: a report by David Dickson on the role of the scientists in Brazil in criticising Brazilian energy policy; and environmental research in the Amazon

correspondence

Fusion first for USSR

SIR,—A short report was published (*Nature* 269, 370; 1977) concerning the "generation of neutrons by thermonuclear fusion using a concentric explosion with an exceptionally high degree of symmetry". The results were obtained by the Polish group headed by Professor S. Kaliski and were announced at an international conference in Prague.

Similar results obtained in the Soviet Union were made public in 1958 by L. A. Artsymovich at the Second International Conference of the Peaceful Uses of Atomic Energy held in Geneva. (L. A. Artsymovich, *Controlled fusion research in the USSR; Proceedings of the Second United Nations International Conference of the Peaceful Uses of Atomic Energy*, 2298, 31, 1958).

As early as 1955, 10^8 neutrons per impulse were generated in our experiments (which were mentioned by L. A. Artsymovich) by focusing converging spherical shock waves on a UD_2T target. In 1963 for UD_3 and gaseous D_2 targets this number was increased to 3×10^{11} . The converging shock wave was formed by implosion of a spherical charge of explosive. In most of the experiments the external diameter of the design was about 70 cm, the mass of gaseous D_2 being about 3×10^{-4} g.

Yours faithfully,

A. S. KOZYREV
V. A. ALEKSANDROV
N. A. POPOV

Leningrad, USSR

Correspondence and papers of Michael Polanyi

SIR,—A major part of Michael Polanyi's papers is now in the Joseph Regenstein Library at the University of Chicago. These are the papers that were in his possession in Oxford, but no systematic effort has been made so far to collect correspondence, manuscripts and memorabilia from people with whom he was in touch. The question as to how to collect this material comes at a time when a biography is being undertaken by Professor W. T. Scott of the University of Nevada in Reno. In the effort to combine the advantages of having original documents available for scholarly use at a centre in Britain, with access to the material by the biographer, and ultimate deposit of copies in the Chicago archive, we wish to make the following request.

We ask anyone possessing such material to make copies, and to send the originals to Miss Cicely Argyle, 12 Sunderland Avenue, Oxford. She will acknowledge receipt, and will deliver the material to the Contemporary Scientific Archives Centre in Oxford. This centre, under the direction of Professor Margaret Gowing, will process and catalogue the material and deliver it for permanent deposit to the Bodleian Library.

The making of copies before entrusting documents to the post is clearly important.

The biographer would appreciate having these copies sent to him, and undertakes to see that when he has made use of them they will finally be sent to the archive in Chicago to fill out that collection. He would also appreciate receiving any recollections and memorabilia, as well as suggestions of other sources of material. His address is: Professor W. T. Scott, Dept. of Physics, University of Nevada, Reno, Nevada 89557 U.S.A.

Yours faithfully,

G. N. BURKHARDT
W. MANSFIELD COOPER
ALISTER HARDY
ROBIN HODGKIN
ARTHUR KOESTLER
DRUSILLA SCOTT
TODD
T. F. TORRANCE
VERONICA WEDGWOOD

Effective trypanocides

SIR,—I was astonished to read the statement by your contributor Hugo Van den Bossche in his article 'Chemotherapy of parasitic infections', (22 June, page 626) that Berenil "is at present the only effective trypanocide left for use in cattle".

The reference given for this information is 'Williamson J., *Trop. Dis. Bull.* 73, 531-542 (1976)'. It is most unfortunate that an erroneous statement such as this should be perpetuated and re-published.

The facts are that several other products are available for the control of bovine trypanosomiasis, including 'Samorin' and 'Novidium' (May & Baker Ltd.), 'Ethidium' and 'Prothidium' (The Boots Co. Ltd.), 'Trypanidium' (S.P.E.C.I.A.) and 'Antrycide' (I.C.I. Ltd.).

Yours faithfully,

J. P. CAVILL

May & Baker Ltd.
Upminster, Essex, UK

Credibility of the anti-nuclear lobby

SIR,—I hope I am not too late to comment on Professor Pearce's thought-provoking contribution to the nuclear debate (20th July, p260).

●A "selective, admonishing and often haughty style" is surely more characteristic of the "anti-nuclear lobby" than of Mr Justice Parker.

●Difficult though Professor Pearce finds it to see how "any sane mortal would perpetrate false claims so as to foist an unsafe industry on an unsuspecting public", this appears to be exactly the opinion of many of the nuclear industry's critics. I also rather suspect that his remark about "the desire to be right and toe the party line" again describes an attitude more characteristic of the critics than of the industry.

●I doubt if anyone would argue that the nuclear power industry never makes

mistakes. But I would like to advance the apparently outlandish idea that the anti-nuclear lobby can make mistakes too.

●Professor Pearce's point about a possible change in the make-up of society invalidating the case for nuclear power is weak on two levels. First, what would we think of an administration which declined to build public housing, schools or hospitals, or even to reform itself, on the grounds that presently prevailing values cannot be assumed to be those of the future? Second, even if society changes world-wide in the direction the critics wish, there will still be a demand (albeit reduced) for central power stations. Is there not then a case for building nuclear plant, and allowing the dearer, dirtier and more dangerous coal and oil burners to be phased out? To forestall a possible objection, I fully agree that a community limited to near the surface of one planet cannot continue to grow indefinitely. But why the optimum level of economic activity should be just that of the developed world in 1978 has never been explained, to my satisfaction at least.

●It is certainly proper to put a case against nuclear power. But the advocates of this case are surely honour bound (as I think Professor Pearce would agree) both to examine this case themselves, and to permit others to do so. It is my distinct impression that many of the present 'antis' do neither now, and would do neither in the future in Professor Pearce's "institutions to debate and evaluate those alternatives".

●While not all the opposition to nuclear power is irrational, a great deal of it certainly is, and it is surely unfair to castigate as "hysterical outbursts" any statement to this effect.

●In the face of some of the arguments put to him, is it not understandable that Mr Justice Parker should occasionally be "perplexed and confused"? For example, reportedly one suggestion was that future energy needs should be met with "cosmic forces". This sort of talk perplexes and confuses even me, and if that makes me a haughty and unfeeling rationalist, so be it.

●Whether the debate becomes a conflict is surely in the hands of the critics as much as of the industry. The death of a demonstrator at Creys Malville in France makes this point sadly dramatic. A recent article in *New Scientist* (3 August, p349) is distinctly ominous, and I for one would welcome Professor Pearce's comments on the kind of tactics hinted at therein.

Lastly because of the tone of the "debate", I had better state that I am a physicist with no professional connection to the nuclear industry. I also hold that complete lack of bias, like perfect social justice, is an unattainable ideal. But, again like social justice, it is very much worth-while to work towards it.

Yours faithfully,

J. F. CRAWFORD

Klein Dottingen
Switzerland

news and views

GABA and benzodiazepine receptors

from Leslie L. Iversen

MINOR tranquillisers of the benzodiazepine series (diazepam = Valium for example) are the most widely prescribed of all psychotropic drugs. The discovery last year (Squires & Braestrup *Nature* **255**, 732; 1977; Möhler & Okada *Science* **198**, 849; 1977; see *News and Views* **266**, 678; 1977) of specific high affinity binding sites for ^3H -diazepam in mammalian brain, possibly representing a novel type of 'benzodiazepine receptor', was thus an exciting breakthrough.

Previous work on this group of drugs had suggested that they might mimic or enhance the synaptic actions of the inhibitory amino acid transmitter γ -aminobutyric acid (GABA) in the brain. At first, biochemical studies failed to reveal any direct connection between benzodiazepines and GABA at the receptor level. Thus benzodiazepines did not influence the binding of ^3H -GABA to its receptor sites in brain membrane preparations, and conversely GABA and related drugs did not influence ^3H -diazepam binding. A series of recent studies, however, now suggests that some interaction may exist between the two types of receptor. Thus, Tallman *et al.* (*Nature* **274**, 383; 1978) report that GABA and the GABA-like drug muscimol cause an increase in the apparent affinity of binding sites for ^3H -diazepam in rat cortical membranes *in vitro*. This effect is blocked by the GABA antagonist bicuculline, which itself lowers the affinity of the drug receptor sites for ^3H -diazepam.

Other studies suggest that, as with enkephalins and opiate receptors, brain normally contains an endogenous ligand for the benzodiazepine sites. Thus, Karobath *et al.* (*Eur. J. Pharmacol.* **49**, 323; 1978) and Marangos *et al.* (*Life Sci.* **22**, 1893; 1978) describe the existence in acid acetone extracts of bovine brain of substance(s) (molecular weight less than 1000) which inhibit the binding of ^3H -diazepam. The specificity of these compounds, however, is not yet clear. The substance described by Karobath *et al.* for example, was present not only in brain but could be detected in even larger amounts in peripheral tissues such as heart and skeletal muscle.

A very interesting proposal has been made by Toffano *et al.* (*Proc. natn.*

Acad. Sci. U.S.A., **75**, 4024; 1978), (see also Guidotti *et al.* *Alfred Benzon Foundation Symposium GABA Neurotransmitters*, 22–25 May, 1978, Munksgaard, Copenhagen, in press; Guidotti *et al.* this issue of *Nature*, page 535), who describe the purification from rat brain of a protein which seems to act both as a ligand for benzodiazepine receptors and as a modulator of GABA receptors. They studied the binding of ^3H -GABA to synaptic membranes from rat brain *in vitro*, and confirmed what others have found (Enna & Snyder *Molec. Pharmacol.* **13**, 442; 1977; Greenlee *et al.* *Life Sci.* **22**, 1653; 1978), namely that high affinity binding of ^3H -GABA is not detectable in freshly prepared membranes but becomes evident only after the membranes have been subjected to exhaustive washing. In fresh membrane preparations only a limited amount of specific ^3H -GABA binding, with relatively low affinity ($K_d \sim 200$ nM) can be detected, whereas after repeated washing, freezing and detergent treatment specific binding increases and a high affinity component ($K_d \sim 20$ nM) is revealed. The supernatant fractions from fresh membrane preparations contained a substance which inhibited high affinity GABA binding when added to washed membranes. Chromatographic purification of this material yielded a 500-fold enrichment of a heat stable protein, with apparent molecular weight 15,000. The possible relation of this endogenous inhibitor of GABA receptors to benzodiazepine receptors stems from the observation that the purified substance is able to inhibit the binding of ^3H -diazepam when brain membranes are preincubated in its presence. Furthermore, incubation of fresh membrane preparations with quite low concentrations of various pharmacologically active benzodiazepines seems to displace the endogenous inhibitor and converts the GABA binding properties of such membranes

to the high affinity type normally seen after removal of the inhibitor protein by washing. Thus, the benzodiazepines might act normally to enhance the actions of GABA at its receptors by displacing an endogenous modulator protein which forms part of a macromolecular complex constituting the GABA receptor-ionophore.

This attractive hypothesis will certainly generate a great deal of interest and further work. It is perhaps surprising that small amounts of the endogenous GABA receptor inhibitor could be detected in non-cerebral tissues such as liver. The relevance of this mechanism to the pharmacological actions of benzodiazepines also rests critically on the correlation between the known pharmacological actions of various benzodiazepines *in vivo* and their ability to displace the endogenous modulator protein from brain membranes; so far only a limited number of such drugs have been tested.

Our understanding of the cellular localisation and molecular nature of the benzodiazepine receptors remains limited. Labelling of benzodiazepine receptors *in vivo* has been demonstrated using ^3H -flumetrazepam (Chang & Snyder *Eur. J. Pharmacol.* **48**, 213; 1978) and ^3H -diazepam (Willamson *et al.* page 533, this issue of *Nature*). The findings of Chang *et al.* (*Proc. natn. Acad. Sci. U.S.A.* in the press) suggest that these receptors are located in large part on glial cells in rat brain. They report that a variety of chemical or surgical lesions which destroy the neuronal elements in corpus striatum or cerebellum of rat brain fail to alter the density of benzodiazepine binding sites in these brain areas. Furthermore, benzodiazepine sites were enriched in subcellular fractions containing glial cells, and like Guidotti *et al.*, Chang *et al.* were able to detect high affinity benzodiazepine binding sites in membranes prepared from rat C6 glioma cells. The postulated glial localisation of benzodiazepine sites, however, is not easy to reconcile with the notion that these sites interact directly or indirectly with GABA receptors, which are thought to be located largely on neurones. The fact that ^3H -diazepam and ^3H -GABA binding sites do not show parallel distributions among brain regions in any case suggests that they are not necessarily linked—and indeed may not even occur on the same cellular elements. □

Leslie L. Iversen is Director of the MRC Unit of Neurochemical Pharmacology, Cambridge.

Anomalous cosmochronology . . .

from Stephen Moorbath and David N. Schramm

FROM the comprehensive sweep of topics at a recent conference on the use of isotopic dating in geology and cosmology*, it is clear that isotopes can shed light on almost every aspect of Earth and Planetary Sciences. Reviews ranging from their use in dating the Universe to their use in deducing the diet of our ancestors indeed provided food for thought.

There has been a revolution in our understanding of the early Solar System as a result of the discoveries of isotopic anomalies in Allende and other carbonaceous chondritic meteorites. The discoveries were led off in 1973 by Clayton and his group at the University of Chicago, who discovered that the oxygen isotopic composition had large variations which could not be explained by simple fractionation and seemed to require an admixture of a different nucleosynthetic component that that of the bulk of the Solar System material. The addition of these non-standard nucleosynthetic components was given a timescale when Typhoon Lee, D. Papanastassiou, and G. J. Wasserburg (Caltech) showed that ^{26}Al was present when objects in the Solar System solidified. Since ^{26}Al was presumably made in a supernova and ^{26}Al has a half-life of only 7×10^5 yr, this seemed to indicate that a supernova explosion had occurred within a few million years of the formation of the Solar System, and had injected freshly synthesised material into the protosolar cloud. With these observations in mind, various astrophysical theorists have developed models for forming the Solar System utilising supernova shocks and grain formation and penetration as a means of getting the anomalies from the supernova into the protosolar cloud.

New isotopic anomalies

At the Snowmass conference, observations of new isotopic anomalies both elucidated and complicated the scenario. Although most of the rocks in Allende have almost normal Solar System isotopic composition, with the exception of oxygen anomalies and excess ^{26}Mg due to the decay of ^{26}Al , it was found about a year ago that two inclusions in Allende, labelled C-1 and EK 1-4-1, were anomalous for almost every element included. In addition, the nature of the isotopic anomalies was different in each inclusion. For ex-

ample, where one particular set of neutron-rich isotopes might be enriched in EK 1-4-1, the same set is depleted in C-1. The discovery of the anomalies in these samples was made by Clayton's group, Wasserburg's group, and G. Lugmair (University of California, San Diego). New and extremely intriguing isotopic information on these two inclusions was presented. In particular, hopes for a simple explanation tying all the anomalies to one type of nucleosynthetic process were complicated by the discovery that in some mineral separates both proton-rich and neutron-rich anomalies were found, whereas in others only one or the other was present. Also, whereas C-1 seems to show more elements anomalous than normal material, it is depleted in the amount of freshly injected ^{26}Al . Since the anomalies in C-1 for the most part tend to be depletions rather than additions, this may mean that C-1 is a 'truer' sample of the protosolar material, and what we now call normal is really a well-mixed sample, including the ejecta from a supernova. Perhaps EK 1-4-1 is a sample in which the supernova ejecta is enriched relative to normal. The question of the composition of the different components which went to make up the Solar System and eventually became mixed together to yield what we call normalised isotopic composition will continue to be worked on, and there are, no doubt, going to be many new surprises.

Universality

The universal nature of the ^{26}Al chronology, and thus its significance, was strengthened considerably by new results reported by J. C. Lorin and M. C. Michel-Levy (Paris) who showed that ^{26}Al anomalies are also present in the Leoville carbonaceous chondrite, whilst J. D. MacDougall (University of California, San Diego) and D. Phinney (Johnson Space Center) showed that the carbonaceous chondrite Murchison also had ^{26}Al enrichment comparable to that in Allende. They also found that some samples of Murchison showed extremely large mass fractionation of Mg isotopes. This large mass fractionation is intriguing because the only other samples that have shown large isotopic fractionation were EK 1-4-1 and C-1.

One point which received some attention was the relation between the 10^6 -yr ^{26}Al time scale and the 10^8 -yr time scale relating the last nucleosynthetic event to produce ^{129}I and ^{244}Pu to the time when xenon daughter products of these two radioactive nuclei

were retained in meteorites. Typhoon Lee and his collaborators at Chicago showed that it may be quite possible for a supernova simultaneously to make the ^{26}Al and the neutron-rich heavy anomalies observed in EK 1-4-1 and yet not produce ^{129}I and ^{244}Pu . On the subject of ^{129}I , one point brought out by A. Zaikowski (University of California, Berkeley) was that in Allende there seem to be at least two separate periods of condensation separated by about 3.7 Myr. That is, the ^{129}I seemed to be included in some rocks 3.7 Myr before others. This shows that some material in a meteorite may be considerably more primitive than other material in that same meteorite. C. Allègre and a team at Paris are trying to utilise the different nucleochronologies and retention times from Pb, Sr, Ar and Xe to show that the time/temperature history of the early Solar System was probably not monotonic, but went through at least one secondary reheating stage for which he set some parameters, but not all.

Hints of possible new results which have not yet been substantiated, but which if true would be extremely exciting, were presented by O. Manuel (University of Missouri, Rolla) who showed that there may be a correlation of Te isotopic anomalies with the strange Xe anomalies found enhanced in some carbonaceous chondrite material by E. Anders and his group at Chicago. Another intriguing suggestion based on preliminary experimental results was that of J. L. McCrumb and his collaborators at San Diego, who suggested that it may be possible for chemical fractionation to produce non-linear isotopic effects. If true, this would have extremely important implications for interpretations of such things as the oxygen isotopic anomaly.

There was also discussion of cosmological timescales as well as the discussion of timescales in the early Solar System. It was pointed out by K. Hainebach and his collaborators that a consistent age for the Universe can be obtained, utilising not just nucleocosmochronologies, but also the traditional Hubble dynamics and globular cluster ages and big bang nucleosynthesis. The age for the Universe where all methods agree is between 13.5 and 15.5 billion yr.

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*The Fourth International Conference on Geochronology, Cosmochronology and Isotope Geology was held at Snowmass-at-Aspen, Colorado, August 20-25, 1978.

... and geochronological anomalies

MANY papers discussed the application of all the available age methods to igneous, metamorphic and sedimentary rocks from all over the world, ranging in age from ancient to almost modern. Finding the oldest terrestrial rocks is a fashionable and prestigious exercise. J. Barton Jr. (Witwatersrand University) obliged with gneisses from the Limpopo belt of southern Africa which yielded a rubidium-strontium whole rock isochron age of nearly 3.8 billion yr, although the uranium-lead systematics of these rocks are highly complex and gave younger ages. At the other end of the geological time scale, the controversy regarding the age of the KBS-tuff, an important marker horizon in the Plio-Pleistocene succession of Northern Kenya, continues unabated. G. H. Curtis and his collaborators (University of California) favour an age of ~ 1.8 Myr, whereas F. J. Fitch and coworkers, from the Universities of London and Cambridge, favour ~ 2.5 million years. Clearly a tuff nut to crack!

The new and exciting samarium-neodymium age method was thoroughly aired by many workers. The geochemical coherence of parent and daughter forms a sharp contrast to the rubidium-strontium and uranium-lead methods and offers the possibility of tracing crustal evolution in certain geological environments which could wholly or partly disturb rubidium-strontium and uranium-lead systematics.

Geochemistry

On the application of initial strontium, lead and neodymium isotopic ratios to petrogenetic studies of igneous rocks and of the geochemical and temporal evolution of the Earth's continental and oceanic crust, and of the upper mantle, most speakers considered that geochemical heterogeneities have existed in the upper mantle for at least 1 to 2 billion years, as judged from isotopic measurements on oceanic basalts. Another issue, still highly controversial, concerns the origin of calc-alkaline lavas (mostly andesites) at continental margins, such as the west coast of South America. Conflicting interpretations of 'anomalous' isotopic and trace-element compositions were proposed by D. E. James (Carnegie Institution, Washington) and by J. R. Lancelot and colleagues (University of Montpellier and United States Geological Survey, Denver). James favours derivation of andesites by massive subduction and partial melting of vast quantities of sediments of continental

origin, whereas Lancelot *et al.* prefer selective sialic contamination with radiogenic strontium of an essentially mantle-derived magma with low $^{87}\text{Sr}/^{86}\text{Sr}$ ratio during fractional crystallisation of the calc-alkaline liquid. This controversy is closely related to the problem of whether the continental crust grows through geological time, or whether its volume has remained essentially constant. I (S.M.) believe that the bulk of isotopic, geological, geochemical and geophysical evidence very definitely favours continental growth, but both arguments will continue for a long time yet. Nonetheless it is now certain that continental igneous rocks frequently acquire an isotopic imprint of the older continental crust through which they make their way to the surface, and this phenomenon can be used to provide constraints for the age and nature of rocks at depth which are not exposed at the surface at that particular locality. P. N. Taylor and S. Moorbath (University of Oxford) reported that the 3.7 billion yr-old sialic craton of West Greenland appears to underlie only a very small part of the voluminous, extensively exposed 2.9 billion yr-old craton. Furthermore, W. Compston and B. Chappell (Australian National Uni-

versity) believe that they have demonstrated that Precambrian continental material was present in the lower crust of southeastern Australia during the early Palaeozoic, so that crust in this region did not originate solely as new oceanic crust at that time.

On the non-radiogenic stable isotope front, the oxygen isotopes as always offer a wide range of fundamental geological applications. F. J. Longstaffe, H. P. Schwarcz and R. H. McNutt (McMaster University) have shown that up to the onset of partial melting the oxygen isotope ratios of Archaean granitoid gneisses reflect the nature of their protoliths. Fortunately, the conclusions closely parallel those drawn from radiogenic isotope studies. Furthermore, the irrepressible H. P. Taylor, Jr. (Caltech), as usual presenting as many new data as all other workers combined, showed how oxygen isotope ratios vary sympathetically with initial strontium isotope ratios in passing from west to east in the great Peninsular Ranges Batholith of Southern and Baja California, and how this must reflect an increasing crustal component in the petrogenesis of this major rock unit.

This was an important and magnificently organised conference. Those geologists who nowadays still ignore, misunderstand and disbelieve constraints imposed by isotopic and geochronological data very definitely do so at their own peril. □

Presynaptic receptors

from S. Z. Langer

THE classical picture of events at nerve endings and synapses has been modified over the past few years by accumulating evidence for the existence of presynaptic receptors for neurotransmitters, in addition to the classical postsynaptic receptors which mediate the response of the effector organ (in the periphery) or of the postsynaptic neurone (in the central nervous system). The presynaptic receptors, found on the outer surface of the nerve endings from which neurotransmitter is released, are thought to be involved in the modulation of the neurotransmitter release evoked by stimulation, by means of a negative feedback mechanism in which a neurotransmitter regulates its own release.

As U. S. von Euler (Karolinska Institute, Stockholm) pointed out in his opening lecture at the First International Symposium on Presynaptic Receptors*, the first indications of the existence of presynaptic receptors can

be traced back as far as 1957 when results on the effects of alpha-adrenoceptor blocking drugs on the stimulation-evoked release of noradrenaline from noradrenergic nerve endings triggered considerable interest (Brown & Gillespie *J. Physiol. Lond.* 138, 81; 1957). It was not until 1971, however, that the hypothesis was formulated that alpha-adrenoreceptors are present in noradrenergic nerve endings and are involved in negative feedback control of noradrenaline release. Since then evidence for presynaptic receptors in the peripheral and central nervous systems has continued to accumulate (see for reviews Langer *Biochem. Pharmacol.* 23, 1793; 1974; *Br. J. Pharmacol.* 60, 481; 1977; Starke *Rev. Physiol. Biochem. Pharmacol.* 77, 1, 1977; Westfall *Physiol. Rev.* 57, 659; 1977).

Evidence for the presynaptic location of the alpha-adrenoreceptors that regulate noradrenaline release has been found in the rat heart. In this tissue there is a significant reduction of ^3H -dihydroergocryptine binding after de-

* Held in Paris on 22-23 July, 1978

generation of the noradrenergic nerve endings induced by the administration of 6-hydroxydopamine. The loss of alpha-adrenoceptor binding sites after chemical sympathectomy indicates that some of these sites are located presynaptically on noradrenergic nerve endings.

Several contributions stressed the fact that there are differences in affinities for agonists as well as for antagonists between the postsynaptic alpha-adrenoceptors that mediate the responses of the effector organ and the presynaptic inhibitory alpha-adrenoceptors that regulate the release of noradrenaline during nerve stimulation. These differences open up the possibility that selective presynaptic agonists or antagonists could be developed to elicit pharmacological actions as a result of effects on neurotransmission. These changes in transmitter release can be brought about through the selective activation or blockade of presynaptic receptors, and might be of therapeutic importance in conditions such as hypertension, depression and schizophrenia.

In addition to the presynaptic inhibitory alpha-adrenoceptors that regulate noradrenaline release in the peripheral and the central nervous systems, presynaptic receptors involved in negative feedback mechanisms for other neurotransmitters have been reported in the peripheral and in the central nervous systems. Evidence for presynaptic inhibitory muscarinic receptors was presented for peripheral as well as for central cholinergic nerve endings. The presence of presynaptic inhibitory dopamine receptors was reported in the rabbit nucleus caudatus and also in the rat striatum. In addition, results were presented which are compatible with the presence of presynaptic inhibitory GABA receptors in the GABA-ergic nerve endings of the rat substantia nigra. It is therefore possible that in addition to the presynaptic receptor systems involved in the autoregulation of the release of noradrenaline, acetylcholine, dopamine and GABA, that presynaptic receptors for other neurotransmitters might be present in nerve endings to subserve the function of the fine regulation of transmitter release upon the arrival of nerve impulses.

In addition to the presynaptic receptors through which the neurotransmitter can regulate its own release, a real mosaic of receptors seems to be present on the noradrenergic nerve endings. Evidence was presented for several presynaptic receptors which are involved in the inhibition of noradrenaline release during nerve stimulation: the muscarinic receptor, the dopamine receptor, the prostaglandins of the E series and in some neurones, the opiate

and adenosine presynaptic inhibitory receptors. Presynaptic receptors involved in the facilitation of the stimulation-evoked release of noradrenaline include beta-adrenoceptors and also angiotensin II receptors. These presynaptic receptors can be activated by other neurotransmitters (acetylcholine) by circulating endogenous compounds (adrenaline, angiotensin II) or to locally produced substances (prostaglandin, adenosine) to modulate transmitter release.

In the central nervous system there is increasing evidence for the modulation of the release of neurotransmitters through presynaptic receptor mechanisms. In the rat cerebral cortex β -endorphin and enkephalin reduce, while GABA enhances the stimulation-evoked release of noradrenaline. In

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addition, the stimulation-evoked release of dopamine from dopaminergic nerve endings can be modified by acetylcholine and GABA. Another example is the inhibition by enkephalin of the potassium-evoked release of substance P. It was concluded that local neurotransmitter interactions involving presynaptic receptor systems may represent a physiological mechanism for the modulation of transmitter release in the central nervous system. Attention was drawn to the fact that presynaptic receptors can be acted on by exogenously administered agonists or antagonists to modulate the release of neurotransmitters induced by nerve impulses. The potential therapeutic relevance of this principle was discussed and it seems that presynaptic receptors in the peripheral and the central nervous system could be considered as target receptors for the development of new drugs. □

Intraplate earthquakes

from Peter J. Smith

MOST of the world's earthquakes occur in narrow bands clearly associated with plate boundaries. To say that the causes and mechanisms of such earthquakes are therefore perfectly understood would be to exaggerate, but at least it is possible to see in general terms that large-scale plate boundary could quite easily lead to violent releases of stress. But what about intraplate earthquakes, of which there is a not insignificant number? By definition there are no intraplate active oceanic ridges, subduction zones or transform faults. So what causes earthquakes away from plate boundaries?

There are several possibilities. Some intraplate earthquakes may be associated with volcanism, and some specifically oceanic shocks registering on seismographs may be due to underwater slumping. Other seismic events seem to represent slippage of local faults. Whether such faults are always as local in cause as they are in geography is another matter, however, for some have probably been left over from the time when their present localities were closely associated with plate boundaries and others may result from stresses in the interior of a plate viewed as a membrane ('membrane tectonics')—stresses that are bound to be influenced by what happens at the edge of the plate.

All three of these causes ('slumping,

fracture zone activity, and possible submarine volcanism') have been invoked in a very general way by Estill and Odegard (*Geophys. Res. Lett.* **5**, 485; 1978) to account for seismicity in the vicinity of the Hawaiian Islands. Estill and Odegard were much less concerned with causes, however, than with the fact that the Hawaiian ridge appears to be more seismically active than previously thought. They report that between July 30, 1976 and March 30, 1977 the Makapuu Ocean Bottom Seismograph, which lies 10 km off the south-east coast of Oahu, recorded no fewer than 359 local events. Most of the epicentres determined (65 in all) occurred on, and in the immediate vicinity of, Hawaii, which is understandable, although two events of $M_L > 4$ took place near Oahu and Nihoa, respectively, well away from the currently active volcanic centre.

But if Estill and Odegard have produced an observational surprise, Campbell (*Geophys. Res. Lett.* **5**, 477; 1978), who is more concerned with cause than with observation, has come up with a theoretical one. As part of a re-examination of the Charleston, South Carolina, earthquake of 1886 (*U.S. Geol. Survey Prof. Paper* 1028; 1977) Long and Champion recently showed that earthquakes in the Charleston area occur in, or very close to, a large mafic/ultramafic crustal intrusion, and Kane has pointed out that there are similar intrusions in several other seismic regions of eastern North America. This has led several people to suggest

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that some local earthquakes may be caused by the stress concentrations set up by such intrusions in the felsic continental plate.

Campbell has now treated this problem (or at least a simplified version of it) analytically. He shows that a vertical intrusion of circular or elliptical cross section is likely to produce stress concentrations only 20–30% greater than the regional stress if, as most workers have assumed, the intrusion has a greater rigidity ($\times 2$ maximum) than that of the rest of the crust. In general, such a stress differential is unlikely to give rise to local seismicity. But if, as Kane suggested, the intrusion has been serpentinised, the picture is transformed. A serpentinised intrusion could have a rigidity up to 10 times lower than that of the surrounding crust, which could lead to stress differentials of up to 200%. Under these circumstances it is quite possible that

earthquakes would occur in the continental plate close to the intrusion.

Of course, it is quite evident that not all crustal intrusions give rise to earthquakes. Campbell speculates that this may be because some intrusions do not have mechanical properties sufficiently different from those of their surroundings (that is, they are not serpentinised enough); because some do not go deep enough, do not have a small enough radius of curvature, and are not oriented appropriately to the regional stress field (as demanded by the model); and because some, though not excluded by these two considerations, may nevertheless simply be too small to produce other than very low intensity events. Crustal intrusions may not account for all, or even most, intraplate earthquakes, but as a possible cause they must certainly now be added to the existing list. □

Drosophila genetics at Szeged

from Michael Ashburner and Peter Lawrence

FOR peripatetic *Drosophila* geneticists the long hot summer ended with a meeting on *Drosophila* biology at Szeged in Hungary*. Less 'molecular' than the recent conferences in Crete (see *News & Views* 275, 364; 1978) it provided an opportunity to meet many workers from Communist countries. In view of the widespread boycott of the Moscow International Genetics Congress this summer, this

opportunity was especially welcome and opened many eyes to work going on in Eastern Europe and the academic centre, Akademgorodok, in Siberia.

Bands and interbands

The problem of the relationship between the 'genes' of *Drosophila* and the bands of the polytene chromosomes, and the problem of the genetic significance of bands and interbands, continues to be a challenge as we heard in a spirited paper from I. Zhimulev of the Soviet Academy of Sciences Institute of Cytology and

Genetics in Akademgorodok. Zhimulev and his collaborators have taken a very small region of the X chromosome of *Drosophila* including the large band 10A1.2, long famous as the site of the eye colour mutant *vermilion*, and attempted to discover other genetic functions coded for by the same band. It would seem that this very large band does include the information for, in addition to *vermilion* (which codes for tryptophan pyrrolase), at least one and possibly three vital loci. All of these complementation groups seem, from deficiency mapping, to be located at the edges of the band, the bulk of whose material appears to be genetically silent.

Zhimulev is a proponent of the idea that bands do not represent structural or functional units but are, in effect, epigenetic phenomena reflecting only the state of gene activity within a tissue. In Zhimulev's view interbands are active genes, a theory suggested by others. This of course is anathema to those of us who thought that Beermann had dealt a death blow to the 'Istanbul hypothesis', for this would imply that the similarities in banding seen between the chromosomes of different tissues reflect only similarities in transcriptional activity, perhaps largely of what are called, not always with due humility, 'house-keeping' genes. To be certain there are other straws in the wind which indicate that perhaps a major re-evaluation of the problem may not be overdue: to mention but two: D. Ribbert (of the University of Münster) reported last year to the Helsinki Chromosome Conference that the banding patterns of the polytene chromosomes from two tissues of the fly *Calliphora* were completely different and now we have evidence from the gene cloners that 'identical' bands (for example in different stocks of *D. melanogaster* or in different, but related species) may differ in their base sequence, at least in part. Clearly the whole problem should be filed under 'keep a watching brief'.

Related to this subject are studies attempting to analyse genetically structures which everyone agrees are active genes—that is to say the puffs of the polytene chromosomes. Two such were discussed at Szeged—the 87A/87C 'heat shock' inducible puffs (J. Gauscz and colleagues, Szeged) and the 2B early ecdysone inducible puff (E. S. Belyaeva & Zhimulev, Akademgorodok). This difficult approach to the study of gene control in *Drosophila* has yet to bear fruit but at

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THE Biological Research Centre of Szeged, known as Hot Spring Harbor on account of the hot, sulphurous, local waters, was founded some 6 years ago and is the showpiece of Hungarian biology. More than 100 workers are organised into four Institutes, housed in modern and well equipped buildings. An important feature of the Centre is that for 5 years it has benefited from a large UNESCO grant, under the United Nations Development Programme, that in addition to allowing it to purchase equipment has enabled sending many of their younger scientists abroad for periods of one or two years. For example the small *Drosophila* and nematode group, under Istvan Kiss, brought together

biologists from various different fields and nearly all have, in effect, been trained during stays in the laboratories of Western Europe and North America. In many cases the close collaboration between these Hungarian scientists and their former colleagues continues and many of the papers presented at the meeting attested to the value, to both sides, of these contacts. More novel has been the programme within the BRC to train scientists from Third World countries — by scholarships many students, who would otherwise often find it difficult to receive such a training, have been brought to Hungary for quite long periods to collaborate with the Hungarian scientists.

*A Workshop on Current Topics in Insect Development was held at Szeged, Hungary on 12–15 September and hosted by the Hungarian Academy of Sciences Research Centre, Szeged. It was supported by UNESCO and the International Cell Research Organisation.

2B several lethal complementation groups have been identified and some alleles seem to show abnormalities in the normal temporal sequence of gene activities inducible by ecdysone in salivary glands. Since the 2B puff is one of the first to respond to the hormone the implications of these observations for the analysis of cascade control reactions during development are clear to see.

Ecdysone receptors

Another topic of enduring interest is that of the general control of development by ecdysone. The meeting was fascinated by accounts of two temperature-sensitive ecdysone-deficient mutants by J. Lepesent (University of Paris) and J. Holden (Queen's University, Kingston), which will be invaluable for the study of ecdysone action. Even more fundamental, however, is the problem of the ecdysone receptors. So elusive have these been that some were beginning to doubt their very existence on anything other than theoretical grounds. The breakthrough by J. D. O'Connor (University of California, Los Angeles) and by Fristrom's group in Berkeley, has now been made. What was needed was a radiolabelled analogue of ecdysone of very high biological activity and very high specific activity. This was synthesised by the tritiated reduction of the phytoecdysone stakisterone C to the very active ponasterone A. Using tissue culture cells O'Connor and his group have characterised a proteinaceous binding activity that does everything an ecdysone receptor should do, at least to those acquainted with vertebrate steroid receptors. Thus the binding shows a temperature-dependent nuclear translocation, a translocation accompanied by a change in sedimentation constant, it is saturable and shows biologically realistic affinity constants to various ecdysone analogues. Moreover derivatives of the original cell line exist which are unresponsive to the hormone and at least some of these seem to lack the receptor. The receptor's purification and localisation to ecdysone-treated salivary gland chromosomes is eagerly anticipated. If current attempts to isolate the ecdysone-responsive genes on

plasmids succeed we are in for an exciting few years.

Sex determination

R. Nöthiger and his colleagues (University of Zurich) are throwing new light on an old problem, at least as far as *Drosophila* is concerned. For years *Drosophila* geneticists have accepted the balance theory of sex determination of Bridges, whereby sex is determined by the ratio between the number of X chromosomes and autosomes, without enquiring too deeply just what this may mean in genetic or developmental terms. Nöthiger suggests that, all organisms, whether man, beast, or green things, possess a 'key gene' whose activation or inactivation is required for sexual development along one or other pathway. In different organisms different methods will be used to control the activity of this key gene: in some (the marine worm *Bonellia*) the signal will be environmental, in others genetic. In *Drosophila* it would be this key gene that responds, by an unknown mechanism, to the X chromosome:autosome balance. The report of T. Cline of Princeton University of a new locus on the X chromosome, which appears to be normally 'on' in females and 'off' in males, and may thus correspond to Nöthiger's proposed key gene, is therefore particularly exciting. Cline has found that if the normal state of affairs is disturbed by mutation, sex-limited lethality results. Nöthiger's laboratory has been systematically analysing the several mutant loci known in *Drosophila* which effect a sex reversal and it was suggested that perhaps the activity of their wild-type alleles is normally subordinate to that of the X-linked key gene.

Cell division and death

The control of cell division and cell death in growing organs remains a mystery, but *Drosophila* geneticists are making a start on its analysis. It has been known for some time that when wild-type cells are generated in a slow growing (*Minute/Minute*⁺) background they grow fast and take over much of the compartment. Morata and Ripoll (*Dev. Biol.* 42, 211; 1975) discovered that when the converse experiment is done—slow growing clones generated in a wild-type background—the weaker cells are completely eliminated. Yet compartments (or indeed whole flies) consisting entirely of *Minute* cells form normally (Ferrus & Garcia-Bellido, *Wilhelm-Roux Arch.* 183, 337; 1977). This phenomenon, termed cell competition by its discoverers, was discussed by P. Simpson (Gif-sur-Yvette) who suggested that the relative growth rate of the cells is the critical factor. *Minute* cells generated in a *Minute*

background, for example, survive. Further, starvation which reduces the total amount of growth, diminishes the effects of competition, rescuing the weaker *Minute* cells. It seems that some kind of cellular struggle which results in survival of the fittest is operating in growing cell populations.

J. Szabad (Szeged) reported results obtained in collaboration with T. Schupbach and E. Wieschaus (University of Zurich). They have made gynandromorphs of *Drosophila* larvae and studied the distribution of male and female epidermal cells. These gynandromorphs are in many respects more valuable than adult ones, as they are a more direct representation of the blastoderm. Thanks to these studies we now know that the ♂/♀ boundary line on the blastoderm is convoluted, and that segmentation coincides with blastoderm stage. For a long time the relationship between the cells on the blastoderm and the gynandromorph fate map has remained uncertain. We now know that each cell on the blastoderm is represented by about 4 sturts on the fate map.

It is always debatable whether conferences are really worth it. In the case of Szeged there can be no question that the formal discussions, and more importantly, the informal ones (especially between colleagues who, for the wrong reasons, so rarely meet) were very worth while. □

The LEP summer study

from a Correspondent

MUCH progress in understanding subnuclear constituents has been made using a new facility for probing the structure of matter at extremely small distances ($\sim 10^{-14}$ cm), namely the electron-positron storage ring. In such a machine beams of electrons and positrons collide head-on producing a single massive photon, which can then pair produce other, heavier particles. This process is quite democratic; any charged particle which exists with a mass less than the equivalent energy of one of the electron beams must be produced with a calculable cross section. Furthermore the production process is 'clean'. The pair of new particles is made alone, devoid of the debris of other subnuclear particles which is always present if the proton beam of a conventional accelerator and a fixed target is used.

The basic constituents of matter seem to be fermions, occurring in families of four, each containing two quarks and two leptons. These fermions

Erratum

In the article 'Illegitimate recombination legitimised' (*News and Views* 274, 213; 1978) the reference (column 3, page 213, line 10) to the 9-base pair repeat generated by the integration of insertion element IS4 and attributed to P. Starlinger, is incorrect. The repeated sequences generated by IS4 have not yet been determined.

interact through vector particles: the photon, charged and neutral massive vector bosons, and gluons which are responsible for the electromagnetic, weak and strong interactions, respectively. So far, experimental evidence for three families of fermions has been found: (u, d, e, ν_e) , (c, s, μ, ν_μ) , (t, b, τ, ν_τ) . Of these, the charmed quark c , the bottom quark b , the heavy lepton τ and its neutrino ν_τ , have only been discovered since 1974. The top quark t , though not yet observed, is confidently expected to be seen in the near future.

It was against the background of these exciting discoveries that some 80 particle and accelerator physicists from Europe and the United States met at the Summer School of Theoretical Physics in Les Houches, France and at CERN, Geneva from 11 to 22 September to study and discuss LEP, the proposed Large Electron Positron storage ring. The energy range of such a machine would extend from the highest energies attainable with the generation of machines now operational (PETRA at DESY, Hamburg, West Germany, see *Nature* **274**, 202; 1978) or under construction (PEP at the Stanford Linear Accelerator Center in the United States), which might be ~ 30 GeV per beam, to energies of ~ 100 GeV and above. Much new and exciting information on heavy quarks and leptons is expected from PEP and PETRA. LEP however is mainly intended to probe the world of the vector particles, in particular the massive bosons W^+ , W^- , Z^0 which we believe are responsible for the weak interaction. Weinberg and Salam have proposed a possible unification of the weak and electromagnetic interactions in which both interactions have the same strength. The observed 'weakness' of the weak interaction results from the large masses of the W^+ , W^- and Z^0 as compared with the massless photon. Such theories predict the masses of the W^+ , W^- and Z^0 from measurements of weak interaction phenomena at low energies. So far the simplest such theory, the Weinberg-Salam theory, agrees with all observed weak phenomena, and predicts masses of ~ 90 GeV for the Z^0 and ~ 80 GeV for the W^+ and W^- . Because of the direct weak coupling of Z^0 to e^+e^- (recently experimentally observed in a beautiful experiment performed at SLAC: SLAC-PUB-2148, to be published in *Physics Letters*) an increase of 10^3 – 10^4 in the rate of interactions is expected if the beams of LEP are tuned to an energy of $1/2 M_{Z^0} = 45$ GeV. W^+W^- pairs of the above mass are also expected to be produced at a reasonable rate for beam energies in LEP of ~ 100 GeV. The observation of such

effects in LEP would mean that nearly a century after the discovery of weak interaction processes, their understanding would be on the same level as quantum electrodynamics (the only exact physical theory of particle interactions we have) and we shall have reduced from four to three the fundamental forces in nature.

In the intervening two years European particle physicists, represented by ECFA (The European Committee for Future Accelerators) under the chairmanship first of G. von Dardel, of the University of Lund, and more recently by M. Vivargent of LAPP, Annecy, have expressed a strong preference for such a large e^+e^- storage ring as the next major European high energy physics facility. During the same period a detailed design study of such a machine has been made (CERN/ISR-LEP/78-17). This design was the basis of the discussions at Les Houches. The storage ring is larger than any existing or planned accelerator, with a radius of 3.5 km. The estimated construction cost of ~ 1000 million Swiss francs is however somewhat less than that of the recently completed CERN SPS proton accelerator. In a preliminary time scale outlined by J. Adams, Executive Director General of CERN, the machine might become operational by about 1988, some 12 years after the start-up of the SPS. The construction of the machine within such a time scale seems possible without significant increase of the current CERN budget of some 600 million Swiss francs per annum provided some existing CERN facilities, by then becoming obsolete, were closed.

Much time was spent at the meeting on alternative 'scenarios' to the simple one described above. What if there were so many neutrinos coupling to the Z^0 that the width is much wider than the 3 GeV now expected? (a suggestion by J. Ellis, CERN). The answer seemed to be that floods of new charged heavy leptons must be seen at LEP. Some theorists (for example M. Veltman of the University of Utrecht) are already looking to even higher energies of more than 300 GeV, where effects of direct $Z^0W^+W^-$ coupling should become discernable. At this energy the currently quite mysterious mass spectrum of the quarks and heavy leptons should become considerably clearer. This is the world of the Higgs scalar boson which in the Weinberg-Salam theory is required to be an observable particle, but of unspecified mass. There were hints that the effects of gluons, the intermediaries of the strong interaction, should be seen particularly clearly in virtual $\gamma\gamma$ collisions, a process with a copious rate at LEP energies. Thus quarks and gluons could

one day possibly be liberated in the laboratory. In his concluding speech S. L. Glashow of Harvard University presented a dazzling catalogue of these alternative scenarios; a cornucopia of subnuclear riches. There have never been stronger arguments for building an accelerator before, and none has been as eagerly awaited as LEP. In the words of the motto chosen by the participants on their last evening at Les Houches, LEP's do it! $\square$

Breeding more protein

from B. J. Mifflin

Now you see it, now you don't. The protein gap much discussed in the late 1960s was largely abolished, at least on paper, by the lower protein recommendations of the 1973 FAO/WHO committee. Subsequently increased controversy has raged between those who argue that there is still a protein gap and those who protest that only calories are lacking. In many respects the arguments are futile and do little for those who have insufficient food which is of poor quality. The FAO/IAEA took a more positive position when in 1968 they organised a meeting to set up a co-operative programme with the GSF to improve the nutritional value of the grains of cereals and food legumes by supporting research programmes in a number of developing countries aimed at modifying protein and amino acid content. This September they convened a conference* to assess the results of the 8 years of the programme and review the present state of knowledge.

The continuing deficiency of adequate nutrition was emphasised in the opening talk by R. Bressani (Instituto de Nutricion de Centro America y Panama, Guatemala City). His results show that by up-grading the protein quality of cereal seed diets, either by using high lysine maize, or by adding legume seed supplements, food intake is increased, infant mortality reduced and the acreage required to provide sufficient protein considerably diminished. Since World Bank estimates indicate that more than a third of the world's population suffers from serious malnutrition the original aims of the programme still seem relevant and

* Organised by the Joint FAO/IAEA Division of Atomic Energy in Food and Agriculture of the International Atomic Energy Agency (IAEA) and the Gesellschaft für Strahlen- und Umweltforschung (GSF) at Neuherberg, near Munich in the Federal Republic of Germany, on 4–8 September, 1978.

worthwhile; the remainder of the conference was chiefly concerned with reporting progress towards achieving those aims.

In the basic sciences considerable advances have been made. The major protein components of seeds are the storage proteins present in protein bodies. These are deficient in lysine in cereals and in sulphur amino acids in legumes. Although there are considerable differences between the storage proteins of legumes and cereals, in both cases they seem to consist of polymorphic families of related polypeptides. In maize, wheat and barley the putative structural genes have been located on various chromosomes and some mapping carried out. Success with *in vitro* synthesis of legumes and cereal storage proteins has been achieved in a number of laboratories and some mRNA has been isolated and cDNA prepared and cloned. These studies should provide a better understanding of the basic mechanisms of protein accumulation in seeds and offer the potential for use of genetic engineering techniques; some of these techniques were discussed in the context of future programmes.

Both as a direct result of the FAO/IAEA programme as well as of studies funded by other agencies, high lysine genes have been found in maize, barley, sorghum and possibly wheat and millet. The current status of the genes in the first three crops was reviewed. Although some progress has been made in understanding the nature of these mutations by determining their chromosome location little is yet known about their biochemical basis other than that the synthesis of the storage proteins is depressed. More information is required as to which of these genes offers the possibility of providing both high quality and high yield. So far the most promising breeding work seems to have been done in sorghum in which yield is not greatly affected, since the smaller size of the high lysine grains is balanced by increased seed number. Very promising results were also reported from CIMMYT, where the use of various modifying backgrounds have led to greatly improved *opaque₂* lines of maize. Although some success has been made with the *Hipoly* gene in barley the lysine content of the lines seems to decrease as yields improve. Besides having normal yields new lysine varieties also have to be accepted by the consumers and meet the normal agronomic requirements, such as disease resistance. Although these criteria have not all been met it must be realised that plant breeding takes a long time and it is necessary to be patient. Some idea of the patience required was demonstrated by the

results of the Nebraska programme of high protein wheat breeding which had been in progress for 20 years before the release of the first variety in the US. This variety, which is now finding acceptance on the basis of its general properties, combines increased protein content and high yield.

In legumes the problems are more concerned with yield and protein quality than with protein content. In a critical and forceful talk H. K. Jain (Indian Agricultural Research Institute, New Delhi) pointed out the large amount of selection pressure and breeding that had been exerted on the cereals in comparison with the legumes. Legumes are not poor in total biological yield but insufficient of it is recovered in the harvested seed. Considerable success has been achieved through mutation breeding and recurrent selection programmes in obtaining improved ideotypes of several legumes with a much better harvest index. As regards protein quality, the problem is to improve the amino acid spectrum of the seed protein as well as to decrease the content of the various adverse nutritional factors present in legume seeds.

The conference ended with a review of the technological aspects of plant use and the role plant breeding and

biochemistry has played and must continue to play. Implicit in this must be an increased realisation that weight of produce alone is not enough and that a direct measure of productivity involves an assessment of the quality of the produce and the uses to which it can be put. When the work reported is viewed as a whole it represents a considerable success particularly in the time frame normally required to bring about stable genetic change and to select improved varieties of crop plants. It is therefore particularly disappointing that this programme is not being renewed with increased funding but, at best, will only continue in a limited way with funds from the Governments of the Federal Republics of Germany and of Sweden. Despite the fact that man's major necessity is food, all of which is ultimately derived from plants, the Plant Sciences remain amongst the Cinderellas, poorly funded and unfashionable. The FAO/IAEA programme has shown what can be done with a fraction of the funds spent on such grandiose technological playthings as supersonic travel. □

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A hundred years ago

M. BOUILLAUD, the once celebrated medical practitioner, who is a member of the Paris Academy of Science, assailed M. du Moncel in the sitting of September 30, and asserted that the phonograph and microphone experiments must be the work of ventiloquists. This fit of incredulity was occasioned by the recital of experiments made with the singing conductors. M. du Moncel asked for a commission of investigation to be appointed, although such accusations are not deserving of any notice, and have, indeed, raised universal ridicule. But the regulations of the Academy forbid any commission to be appointed to pronounce on the works or communications of members. Another curious scene took place at the sitting of last Monday. M. du Moncel presented to his colleagues, the "condensateur chantant," which had been

exhibited on the previous Saturday. He retired to the room of the Académie Française, in company with M. Faye, closed the door and sang. His voice was heard coming from a number of sheets of paper, in which six sheets of tinfoil had been inserted, and connected with the wires of an induction coil. M. Bouillaud was obliged to retreat from the position he had taken at the sitting of September 30. He made no allusion to the accusation of ventiloquism, but read a long quotation from Descartes, to show that "even if a speaking machine had been constructed, it could by no means be considered as a thinking machine." He said that speaking was not only a mechanical action, but also an intellectual work, so that neither the phonograph nor the singing condenser could be regarded by any means as really speaking! The whole assembly, in spite of its usual gravity, burst into roars of laughter. M. Milne-Edwards, who spoke at the previous sitting, said with much propriety, he should not have answered M. Bouillaud if he had understood such was his issue. Unfortunately he had understood, as everybody in the assembly did, that M. Bouillaud questioned the honesty of the experimenter.

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review article

Exploration of the continental basement by seismic reflection profiling

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The structure of the continental crust and uppermost mantle is being explored with higher resolution than previously attained through application of the seismic reflection profiling technique of the petroleum industry. Crustal structure is diverse, and features such as faults, unconformities, magma bodies, and the crust–mantle boundary have been delineated.

SOME four billion people derive their livelihood from this planet, so society's need for a thorough understanding of the Earth and a comprehensive inventory of potentially accessible Earth resources seems obvious. Yet except for mining prospects and districts, and the sedimentary basins that are the habitat of petroleum, there is little detailed or direct knowledge of subsurface Earth structure and composition at depths greater than a few tens or hundreds of metres. In most areas, deep rocks of the crystalline basement can be described only in general terms based on indirect evidence. Their genesis and evolution remain largely unknown.

The reasons for this remarkable state of affairs are partly economic and partly historical and organisational. They are also partly technological although there are means for improving substantially, if not ideally, our knowledge of the crystalline basement. Better mapping and improved field and laboratory studies of surface rocks, drilling for informational purposes, study of xenoliths of basement origin, and further application of a variety of geophysical methods are all feasible and promising. The article focuses on potentially the most productive and the most sophisticated geophysical technique—seismic reflection profiling—and its application to studies of the basement. This does not mean that other methods are not valuable; in general, they provide complementary information. The seismic reflection profiling technique discussed here should not be confused with the seismic refraction technique that is conventionally used for exploration of the crust, or with the Russian DSS method, which uses both refraction and wide-angle reflections.

During 1976 world expenditure on seismic reflection profiling was well over one billion dollars. Essentially all of this money was spent on exploration for petroleum. Continuing effort on this scale by the petroleum industry has resulted in, and is the result of, development of the technique of echo ranging in the Earth to a very high level of sophistication. In recent years, the industry has produced such innovations as non-explosive sources, digital field recording for movable listening arrays of thousands of detectors, computer processing of immense quantities of digital data, and powerful new methods of analysis and interpretation that have greatly enhanced understanding of the stratigraphy and structure of sedimentary basins. Modern reflection profiling bears little resemblance to the simple one shot—one detector method initially employed in the 1920s.

Within the past few years in several different countries, successful attempts have been made to apply these modern

techniques, with appropriate modifications, to exploration of the continental basement and hence greater depths (for a review see ref. 1). Here, the term 'basement' refers to crystalline rocks typically lying below the sedimentary veneer that is some 0–10 km thick. The continental basement rocks of interest here extend through the base of the crust at some 35–45 km and into the underlying mantle to depths of 50 or so km, the current limit of the method. Although rocks of the deep continental basement have been explored with some success previously using various geophysical techniques, the new efforts based on reflection profiling offer far greater resolution of structural detail and much better information on other properties that are currently unknown or poorly known. Because of the potential of this much improved, yet rarely applied, observational capability in seismology, the potential of other geophysical and geological methods, the great extent of the basement, and the direct and informative relation of the deep continental basement to the near-surface rocks, the continental basement must be considered one of the major scientific frontiers of modern geology.

The method

The principle of the seismic reflection profiling technique is deceptively simple. A disturbance at or near the surface of the Earth generates elastic waves that propagate through the Earth and are detected and recorded by sensors at the surface near the source. Although other kinds of waves are sometimes useful, most information comes from the compressional waves that travel along near-vertical paths and are partially reflected at buried sub-horizontal interfaces between rocks of contrasting impedance. The travel time of a pulse propagating along such a path is perhaps the prime information gathered, but the amplitude, phase, frequency content, and apparent surface velocity of the signal are also valuable.

The source and receivers are moved together along a line and the experiment repeated at closely spaced intervals so that subsurface reflecting horizons may be traced, and a profile or section obtained in a similar manner to that of a marine echo sounder. The basic travel-time data must be ultimately converted to depths and reflectors properly positioned; hence it is necessary to know or to measure the spatial distribution of velocities along the profile. One common method for velocity measurements uses reflected waves travelling along paths that are somewhat off the vertical. Thus, a single source might be detected and recorded by many sensors distributed over

distances ranging from near zero to significant fractions of the depth of the deepest reflecting horizon.

Furthermore, because the sources generate kinds of waves (surface waves, shear waves, waves with complex paths, diffracted waves) in addition to the nearly vertical travelling compressional waves that are normally the signal, arrays of many source elements and arrays of many receivers are commonly used to discriminate against the unwanted waves as well as background noise. Some data are also collected off the line, to permit the waves propagating out of the plane of the section to be identified.

In practice, there are numerous variations of the technique which depend on a large number of factors, such as local terrain, local propagation phenomena, economics, available hardware, characteristics of the particular target, and noise conditions.

As an example, a field procedure sometimes used in the COCORP project is described in some detail in the following. (COCORP is consortium for continental reflection profiling, and the project is the major US effort to apply the seismic reflection profiling technique of the petroleum industry to study of the deep basement of the continents. The procedure represents a compromise among various factors including acquisition of maximum possible information, economics, and logistics.)

COCORP uses the VIBROSEIS technique developed by the Continental Oil Company. Instead of an impulse generator such as an explosion, weight drop, or compressed air gun, a truck-mounted vibrator serves as a source element. Each vibrator imparts a peak vertical force of some 13 tons over about 20 ft² of the Earth's surface; the force is near-sinusoidal in time but of slowly varying frequency. The signal thus resembles a radar 'chirp' in character, though not in frequency. Typically the duration of vibration is 20 s, and the frequency varies linearly from 8 to 32 Hz over the interval. Cross correlation of the recorded signal with the input produces a record resembling that from an impulsive source, subject, of course, to restrictions of bandwidth and other properties of the VIBROSEIS source and the propagation. The advantages of the method include flexible and precise control of source character, elimination of drilling to implant explosives, avoidance of legal restrictions on explosives and the explosive hazard, and efficient coupling of source energy into elastic waves without great loss through rupture or other non-linear effects near the source. VIBROSEIS has been used widely and successfully on land by the petroleum industry for many years.

In a typical COCORP survey, stations in the field are spaced at 100-m intervals. Five vibrators operate synchronously 16 times at equally spaced intervals between stations, and the resulting signals are appropriately summed. Thus, 80 sweeps are injected into the earth per station interval and combined to form a source array 100 m long.

The receiving array includes 96 stations and is thus almost 10 km long. At each station a small spread of about 30 detectors, spaced to discriminate against natural and source-generated noise, is summed and recorded as one of 96 channels. Once the 80 sweeps at one station are recorded, the sources and the 3,000-element listening array are, in effect, moved 100 m along the line and the entire process repeated. Thus, along a line a few tens of kilometres in length, information travelling along tens of millions of rays is used in the analysis, a high level of redundancy that can be very advantageous. The data are recorded digitally in a form that will accommodate a very large dynamic range.

A further advantage is gained by computer processing the data. A wide variety and large number of methods including frequency and wavelength filtering, construction of synthetic seismograms, proper spatial positioning (migration), beam-forming, and various signal enhancement techniques are commonly applied depending on the particular problem. One powerful method, called common depth (or common reflection) point stacking, is based on combination of data from rays incident upon a particular reflection point at slightly different angles. Under favourable conditions signals are much enhanced.

COCORP typically uses 12, 24 or 48-fold common depth point stacking. In the sense that information from segments of many rays passing through a common zone is combined, the method resembles X-ray tomography.

Although seismic reflection data appear in other useful forms such as velocity profiles and contour maps, the end product is typically a section with distance as the abscissa and travel time, or depth, as the ordinate. For the casual observation, and to a first approximation, this may be thought of as a section through the Earth. It is misleading to carry this view too far, however. A detailed interpretation of the data requires an understanding of wave propagation, field procedures and data processing, and a knowledge of related geological and geophysical data for the area. This report is too brief to present detailed analysis or interpretation of data. Instead two sections are shown and described in general terms, and some conclusions based on studies to date are presented after a discussion of current efforts to explore the continental basement. (For a more detailed description of seismic reflection profiling techniques and interpretation as found in the petroleum industry see refs 2-5.)

Seismic exploration of the continental basement

A proper strategy for exploring a major frontier of the Earth sciences must include a judicious blend of several factors including the nature, scope and scale of the scientific problems, capability of the method, economics, expertise of personnel, and logistics. In an effort to achieve this blend, the COCORP project operates as follows. Many scientists from industry (largely the petroleum industry), government agencies, and universities participate in committees to advise on technical, scientific, and administrative problems. Cornell University acts as the operator or prime contractor. The field work is conducted by large geophysical contracting companies. Data are processed through routine stages, and available at modest cost, so that further specialised processing and interpretation are facilitated.

The choice and nature of sites for such surveying are critical. Six sites have been surveyed to date in the US. The first site in Hardeman County, Texas, was selected for favourable seismic and field conditions in initial testing of the method, and with little regard for the geology. Later sites, two in the Rio Grande Rift near Socorro, New Mexico, one crossing the boundary fault of the Wind River Mountains in Wyoming, one across the Franciscan basement-continental basement boundary in the Great Valley of California, and one across the San Andreas fault in California, were selected because of geological problems of special interest. A recognised problem of regional or crustal scale, as opposed to local or surficial scale, is found at each of these sites. Typically, a few lines of reflection profiling totalling some tens of kilometres have been carried out at each site. In all cases the results have been highly encouraging. In the longer term, however, it is planned that longer lines traversing the entire continent will be explored, so that problems of still larger scale will also be investigated. Applying this method of exploration to studying the modern basement is so new, and the region to be studied so vast and varied, that one must anticipate a period of rapid learning and development in the early stages of the project.

Two examples of the data

Figures 1 and 2 show two sections obtained by the methods described above for the locations in the Rio Grande Rift area^{6,7} indicated by 1 and 2A in Fig. 3. The reproductions do not do justice to the original data but some of the major features can be seen. The abscissa for Figs 1 and 2 is the distance in kilometres times 10; the ordinate is two-way travel time. Multiply by three to convert time to approximate depth in kilometres.

In Fig. 1, the upper 1-1.5 s of data for the western two-thirds of the profile show nearly horizontally stratified sediments of the rift valley. The sediments are broken into horsts and grabens by normal faults. It is only this portion of the section that would be

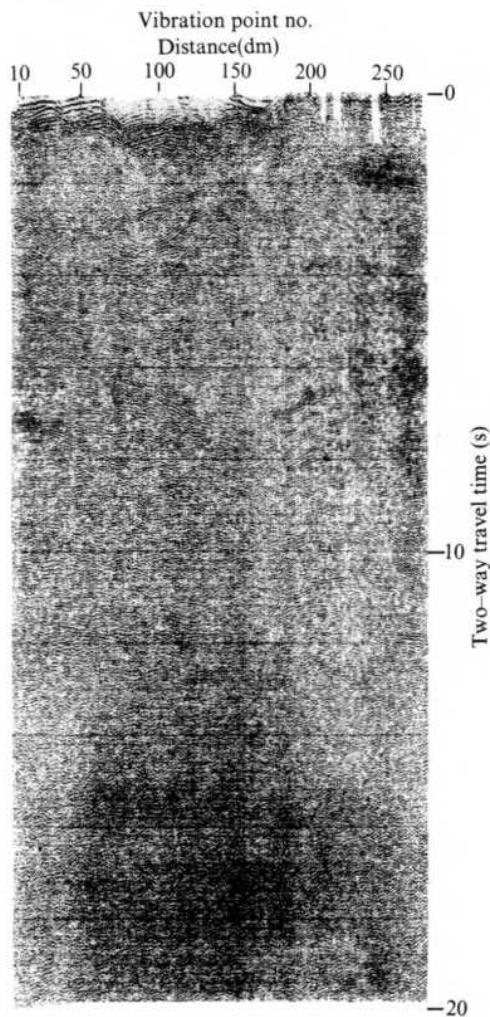


Fig. 1 Seismic section for line 1, Rio Grande Rift (see Fig. 3).

studied in normal petroleum exploration, and in much greater detail. Below these sediments is the crystalline basement which outcrops east of the bounding fault as seen at about station 180. Some shallow Palaeozoic sediments that overlap the basement at the eastern end of the section are indicated by events at <1 s.

Principal interest here is in information from greater depths. In some areas of the section, such as from ~ 1 to 3 s and between stations 10 and 75, there is a paucity of reflected data as indicated by the lack of short horizontal lineups. The rocks of such zones must be relatively free of acoustic heterogeneities. In this same range of times near the centre of the section are some horizons that dip to the west and may be faults. To the east (right) are additional basement structures.

Beneath the bounding fault through most of the record there is a zone lacking in reflected energy, possibly because of intrusives associated with the boundary. To the east (right) considerable structure in the basement is apparent, and at ~ 12 s there is a strong reflector that, based on time, corresponds to the top of the mantle. This reflector cannot be traced across the entire section, although it might be correlated with reflectors to the west at about the same time.

There is little reflected energy on this section at times greater than 12 s. At the western end of the profile there is a strong reflection at 7 s with an associated diffraction. This event corresponds to a horizon identified as the top of a magma body by Sanford⁸. Other profiles in the Rift show additional evidence for this mid-crustal body of molten rock. (For more detailed discussion of these data, see ref. 7).

Figure 2 shows a section for line 2A (Fig. 3) that begins slightly south of line 1 and continues southwards. Some of the major features are (1) the sediments showing much less structure than

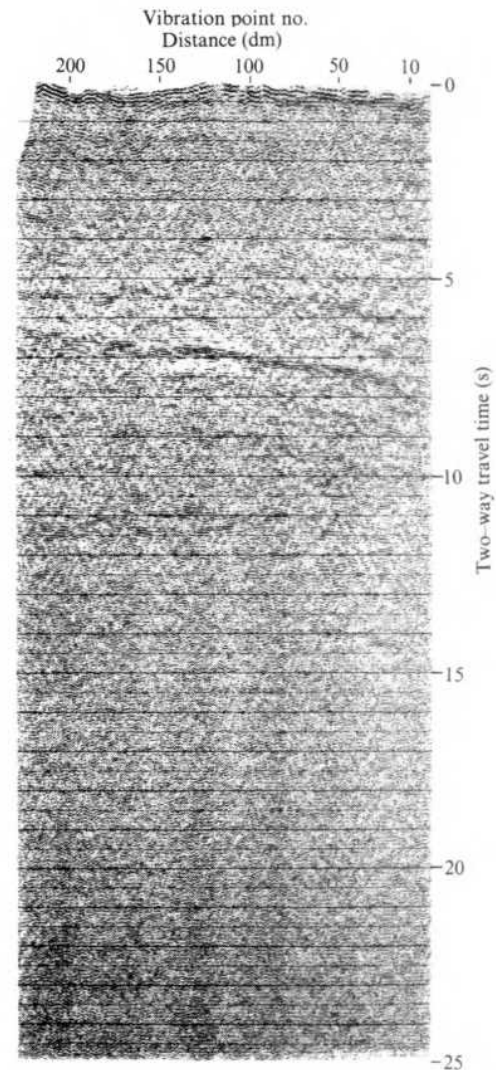
is the case for line 1 because line 2A is more nearly parallel to the geological structure, (2) a transparent zone in the upper basement between the base of the sediments and almost 4 s; (3) a strong reflector corresponding to the magma body at about 7 s and dipping to the north; and (4) a zone of reflected energy between about 11 and 12 s presumably corresponding to the top of the mantle.

The above qualitative and cursory descriptions obscure the great potential for obtaining more quantitative information by careful study, computer modelling, and other forms of analysis, but they provide a first impression of the type of data now available on the crust and illustrate the much improved resolution and vast potential of the technique.

Although seismic reflection profiling of the deep continental basement is relatively new, and only a tiny fraction of the basement has been surveyed at present, some conclusions can be drawn, although they must be held subject to revision as more data are acquired. The following points are based largely on COCORP experience, but they are compatible with similar observations elsewhere (see ref. 1 for a review and citations)^{6,7,9-13,16}.

(1) The continental basement at any particular site some tens of kilometres in dimension is typically heterogeneous on the scale of less than one or a few kilometres. Reflections from within the basement are only infrequently continuous for more than a few kilometres. Diffractions, reflection character, and transparent zones further attest to this heterogeneity. While layered models such as the classical but already outdated granite-basalt crust or

Fig. 2 Seismic section for line 2A, Rio Grande Rift (Fig. 3) for coordinates.



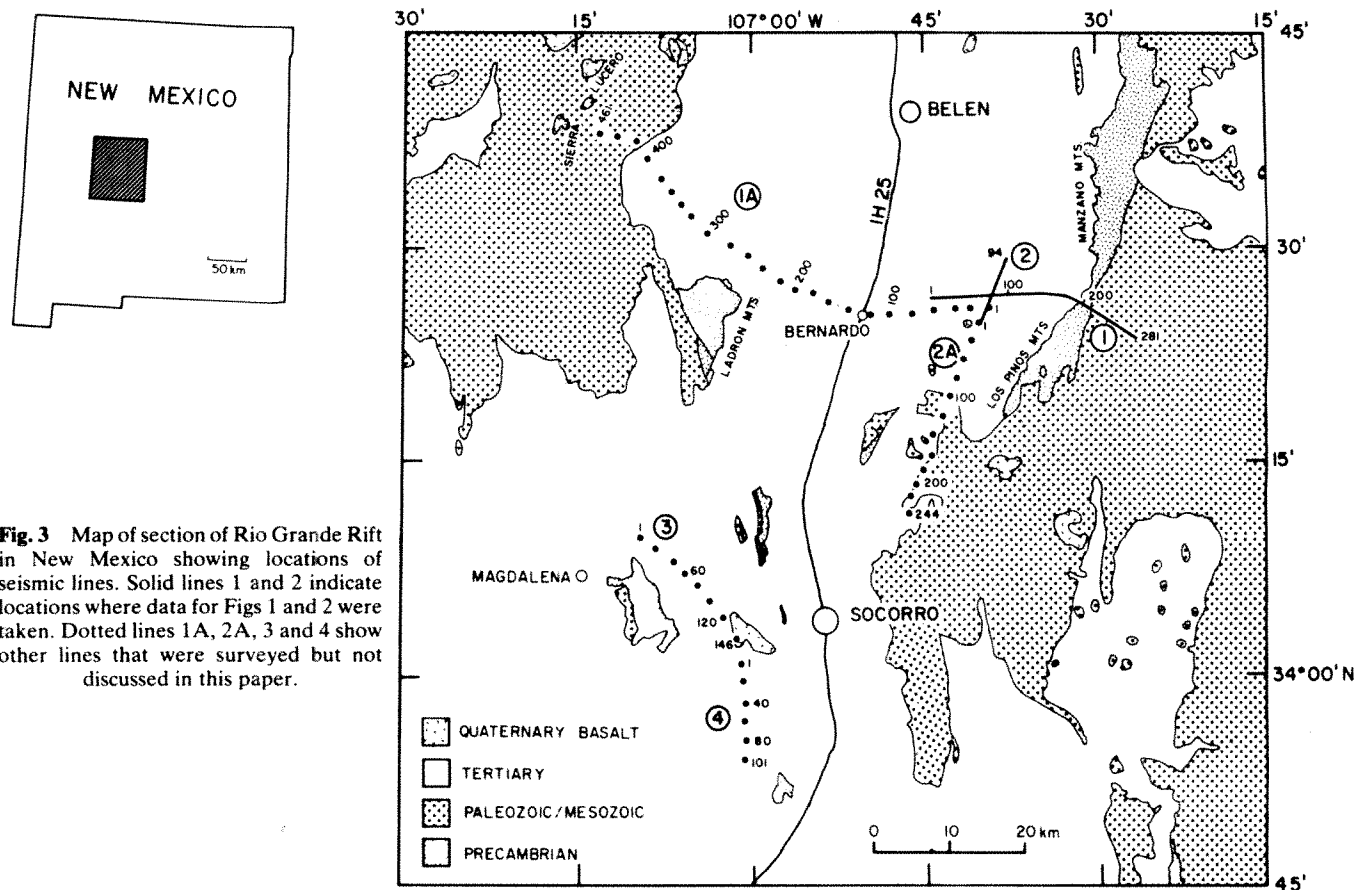


Fig. 3 Map of section of Rio Grande Rift in New Mexico showing locations of seismic lines. Solid lines 1 and 2 indicate locations where data for Figs 1 and 2 were taken. Dotted lines 1A, 2A, 3 and 4 show other lines that were surveyed but not discussed in this paper.

those based on seismic refraction data may be useful for interpretation of some seismic data, they can only be approximations in a gross sense to the geology of the crust. The deep crust seems to be, not surprisingly, like rocks with a history at depth but which are now at the surface (see also ref. 14).

(2) On a larger scale, there is great variation from site to site in the character of the seismic reflection profiles; consequently such variation must exist in the basement geology. With the exception of some features of the seismic profiles in the Rio Grande Rift, many of which show the magma body at a depth of about 18–20 km and some consistency at Moho depths, there is little similarity and great difference in seismic sections from one site to another. In Hardeman County strong diffractors are found in a quantity not observed elsewhere, and there are also shallow basement reflectors of unusual length of continuity, at least 17 km. The San Andreas fault in California shows up as a series of diffractors to depths of ~10 km, while deep reflectors are discontinuous across the fault zone. In the Wind River Mountain area the major thrust can apparently be traced to depths of almost 25 km. Many other deep reflectors corresponding to faults or other horizons may be seen. The Great Valley line shows fewest deep reflectors, perhaps because the Franciscan basement is so badly deformed. At any rate, there is little similarity from one site to another and ample evidence for de-emphasising universal models of the continental crust. It follows therefore, that a wealth of new knowledge of the structure of the crust must be available using the reflection technique.

(3) There is no evidence for a single consistent mid-crustal reflecting horizon corresponding to the Conrad discontinuity, a term that should probably be abandoned.

(4) In existing data the Mohorovicic discontinuity does not generally appear as a single simple reflector. Instead there is commonly a band of reflected energy. A possible interpretation is the laminated sort of boundary proposed by Meissner¹⁵ and perhaps suggested by exposures of the lower crust–upper mantle in the Ivrea zone.

(5) With the abundant evidence from geology and geophysics indicating heterogeneity throughout the crust, it would be remarkable if the mantle directly below the crust were homogeneous.

(6) At particular sites the method reveals features of the basement that, in nature, are geological (as opposed to geophysical) such as active magma bodies, faults, unconformities, folds, intrusions, and perhaps brecciated zones, as well as boundaries between rocks of different acoustical impedances. Detailed discussions of such sites can be found elsewhere^{9–13,16}.

(7) Continued application of the seismic reflection profiling technique including modern developments should provide a wealth of new knowledge and lead to a much enhanced understanding of the continental basement, a major frontier of modern earth science.

The results from the COCORP project depend on numerous participants from universities, government laboratories, and industry. This research was supported by the NSF.

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articles

Effects of intense stratospheric ionisation events

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High levels of ionising radiation in the Earth's stratosphere will lead to increased concentrations of nitrogen oxides and decreased concentrations of ozone. Changes in the surface environment will include an increased level of biologically harmful UV radiation, caused by the ozone depletion, and a decreased level of visible solar radiation, due to the presence of major enhancements in the stratospheric concentration of nitrogen dioxide. These changes are studied quantitatively, using the passage of the Solar System through a supernova remnant shell as an example. Some of the potential environmental changes are a substantial global cooling, abnormally dry conditions, a reduction in global photosynthesis and a large increase in the flux of atmospheric fixed nitrogen to the surface of the Earth. Such events might have been the cause of mass extinctions in the distant past.

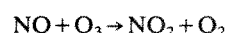
It has been proposed that¹ the observed correlation between extinctions of certain species of marine microorganisms (radiolaria and foraminifera) and geomagnetic polarity reversals could be explained by the occurrence of intense solar flares. Energetic solar protons ionise the Earth's stratosphere, producing nitrogen oxides as a by-product, and thereby depleting the ozone layer that shields the surface of the Earth from lethal solar UV radiation². At present the geomagnetic field confines the direct effects of solar protons to latitudes higher than about 60°, but during the brief periods of weakening or disappearance of the field accompanying a polarity reversal the global effect would be considerably more severe.

The history of life on Earth has been punctuated by widespread and apparently sudden (on the geological time scale) extinctions affecting both terrestrial and marine animals³. Examples of such massive extinctions, each involving a significant proportion of all species existing at the time, occurred at several of the major geological boundaries. The best known is probably the terminal Cretaceous extinction, which occurred about 65 Myr ago, but the fossil record has revealed several other extinction events of comparable severity, and suggests that

they have occurred at approximate intervals of 10⁸ yr. Various mechanisms have been proposed to account for these extinctions, including climatic changes, increasing ecological competition from newly evolving species, changes in sea level, disease epidemics, poisoning by volcanic emissions, increased radiation levels from nearby supernova explosions, and several others. None has proved entirely satisfactory, however, and it seems natural to ask whether the solar-flare mechanism proposed to account for the minor extinctions associated with geomagnetic polarity reversals could also account for these much more catastrophic and massive extinctions that have tended to mark many of the major boundaries of the geological record.

Solar flares

Reid *et al.*¹ discussed principally the increased UV dose received at the surface of the Earth as a consequence of the depletion of stratospheric ozone associated with hypothetical intense solar flares. Their calculations indicated that a solar flare 100 times more intense than that of August 1972 (the most energetic observed over the 20-yr period for which observations exist) would cause a global ozone depletion of about 50% and would increase the biologically effective UV dose received at the Equator by a factor of about 2.5, assuming that the flare occurred during the 10³–10⁴ yr duration of a geomagnetic polarity reversal. Although such an event would have serious enough consequences for living organisms, there is a second environmental effect that was mentioned only briefly. This is the reduction in solar radiation reaching the surface of the Earth in the visible part of the spectrum due to absorption by stratospheric NO₂, whose absorption cross section reaches a peak value of 6 × 10⁻¹⁹ cm² near 400 nm wavelength⁴. NO₂ is formed principally by the ozone-destroying reaction



and absorption of solar visible radiation provides a routine method of measuring the NO₂ content of the atmosphere^{5,6}. The calculations mentioned above indicated that the vertical NO₂ column content would become as large as 6 × 10¹⁶ cm⁻² during

daytime conditions several months after a solar flare 100 times more intense than that of August 1972. The consequent decrease in solar radiation reaching the surface at 400 nm wavelength for an overhead Sun would be about 3.5%, increasing to 7% for a solar zenith angle of 60°. The effect on the integrated solar radiation at the surface would be offset to some extent by the accompanying increase in UV radiation resulting from the destruction of ozone, but significant changes in the Earth's radiation budget, with accompanying changes in lower-atmosphere circulation, could be expected.

As with all suggested mechanisms, however, there remains a large element of speculation that is difficult to remove. Our knowledge of the flare mechanism itself is not sufficient to say whether energetic-particle fluxes 100 times more intense than those of August 1972 can ever be expected, even once in 10^8 yr, or whether there is some absolute limit beyond which a flare cannot go. Given the occasional occurrence of such super-flares, the hypothetical climate change runs up against the problem that the residence time of flare-associated NO_x in the stratosphere is only a few years, probably not long enough to allow the enormous heat reservoir in the oceans to adjust to the new conditions and produce a true climatic change.

Supernova explosions

The degree of speculation is reduced if we consider nearby supernova explosions as a cause of intense stratospheric ionisation instead of solar flares. The suggestion that mass extinctions could have been caused by supernova outbursts is an old one, and was revived by Terry and Tucker⁷, who discussed the potential radiation hazards of such an event. Since then, other consequences of nearby supernova explosions have been suggested. Russell and Tucker⁸ specifically raised the possibility of a climatic change associated with such an event in connection with the terminal Cretaceous extinctions, though without discussing any mechanism. Ruderman⁹ and Whitten *et al.*¹⁰ discussed the depletion of stratospheric ozone expected from nearby supernova explosions, and Hunt¹¹ has recently estimated the surface temperature change caused by this depletion.

Clark *et al.*¹² have suggested that a supernova is likely to occur within 10 pc of the Solar System approximately once in 10^8 yr during passage through one of the spiral arms of the galaxy. At a distance of 10 pc, the Solar System would probably lie within the supernova remnant (SNR) shell for a period of several hundreds of years. There is growing evidence that SNRs are the source of galactic cosmic rays¹³ and that the cosmic rays are accelerated continuously during the lifetime of the SNR. This point of view is

consistent with indirect evidence from the radio emission of SNRs that they contain a cloud of cosmic-ray particles with a total energy of the order of 10^{50} erg, leading to a cosmic-ray flux of the order of 100–1,000 times the present background level.

Model of stratospheric photochemistry

We have used a one-dimensional time-dependent model of stratospheric photochemistry¹⁴ to study the effect of such enormously increased cosmic-ray fluxes on the total solar radiation penetrating the stratosphere. The model calculations were started with initial conditions corresponding to global average present-day conditions, and with all photochemical mechanisms appropriate to a latitude of 30°. The enhanced cosmic-ray fluxes began abruptly on 1 January, and were kept at a constant level for the duration of the calculation, which was taken as 5 yr.

Figure 1 shows the annual average values of total vertical ozone column content for cosmic-ray fluxes 100 and 1,000 times the present value, and indicates that a steady state has been reached after about 5 yr. Total ozone depletions amount to 64% and 89%, respectively, and are considerably larger than those calculated by Whitten *et al.*¹⁰ The differences apparently arise from differences in the model parameters, and in particular in the vertical eddy-diffusion profiles. All model calculations can only be approximate for such major perturbations, however, as it is impossible to estimate the effect of major compositional changes on transport coefficients. Figure 2 shows the corresponding values of NO_2 vertical column content, again indicating a near approach to a steady state after 5 yr, with increases from the present level of $4 \times 10^{15} \text{ cm}^{-2}$ by factors of 13 and 130, respectively.

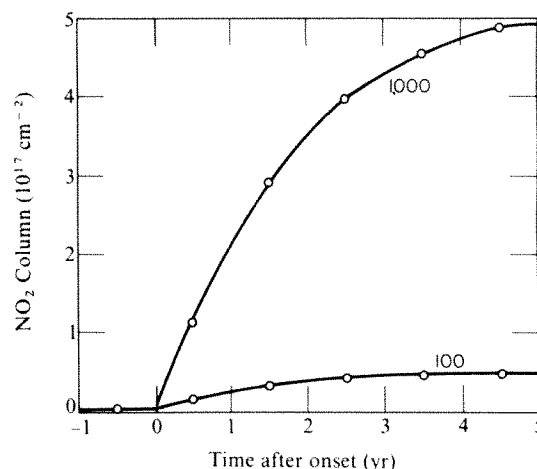


Fig. 2 Vertical column content of NO_2 as a function of time after the onset of enhanced cosmic-ray fluxes.

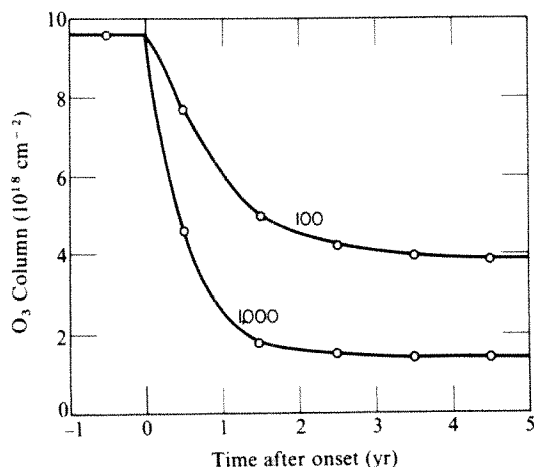


Fig. 1 Vertical column content of ozone as a function of time after the onset of enhanced cosmic-ray fluxes.

Figure 3 shows the annual average steady-state height profiles of O_3 and NO_2 concentration for present-day conditions and for the same two cases of enhanced cosmic-ray flux. Concentrations below 10 km have been omitted because the appropriate tropospheric and surface sources are unknown; their effect on the stratospheric concentrations is negligible. The major depletions of O_3 and enhancements of NO_2 occur in the lower stratosphere, where the photochemical lifetime of ozone is long, and where cosmic-ray ionisation peaks. For the case of a 1,000-fold increase in cosmic-ray flux, the NO_2 concentration approaches that of ozone near 20 km.

As mentioned above, the model calculations are carried out for 30° latitude conditions, and can be taken to represent a global average. In estimating the effects at latitudes other than 30°, we assume that the stratospheric composition is the same everywhere. This assumption would, of course, be invalid if we

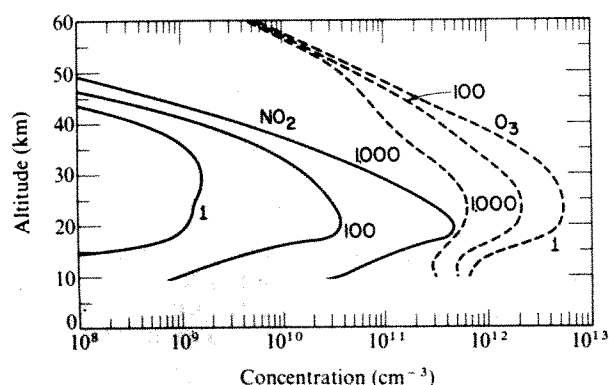
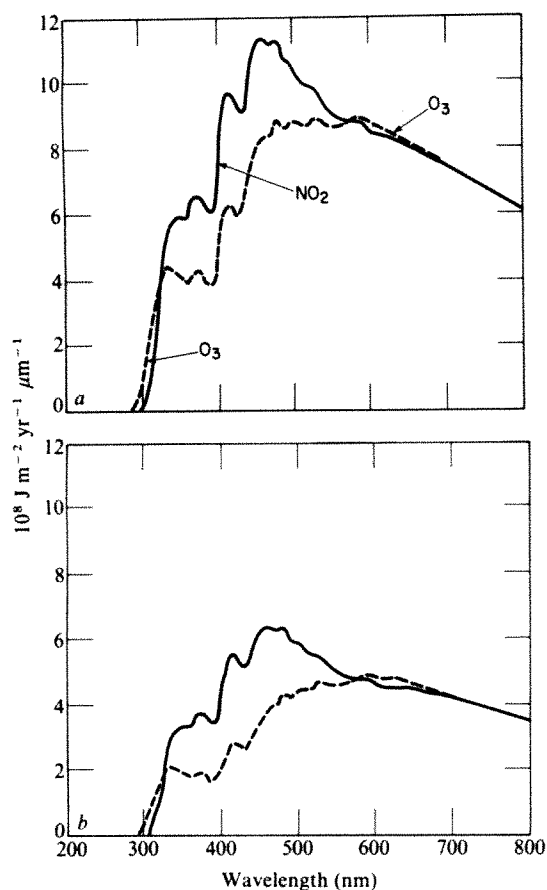


Fig. 3 Vertical altitude profiles of O_3 and NO_2 for present conditions (1) and for enhanced cosmic-ray fluxes (100 and 1,000).

were concerned with absolute values, but it allows us to estimate approximately departures from normal conditions. Figure 4a and b shows the spectral distribution of direct solar radiation penetrating the stratosphere throughout one year for latitudes of 0° and 60° , respectively. The solid curves represent present-day conditions, and the broken curves refer to steady-state (that is 5 yr after onset) conditions for the case of a 1,000-fold cosmic-ray enhancement. Modifications to the spectrum occur in three distinct spectral regions: the annual solar radiation is increased in the near UV, at wavelengths shorter than 325 nm,

Fig. 4 a, Direct solar radiation penetrating the stratosphere at the Equator integrated over one year (solid curve, present-day conditions; broken curve, steady-state conditions for a 1,000-fold cosmic-ray enhancement). b, Same as (a) for latitude 60° .



due to the decreased absorption in the Hartley and Huggins bands of ozone; blue and green visible radiation (325–570 nm) is substantially decreased, due to the increased absorption by NO_2 ; longer wavelength visible radiation is slightly increased, due to the decreased absorption in the Chappuis bands of ozone. The net environmental effects are (1) an increase in the biologically effective dose received by living organisms at the surface; and (2) an appreciable decrease in the total integrated solar radiation available for heating the lower atmosphere and the surface.

Figure 5 shows the fractional change in annual integrated direct solar radiation penetrating the stratosphere at the indicated latitudes for the two cosmic-ray flux enhancements. In the case of the 100-fold enhancement, the integrated radiation is increased by a few tenths of a per cent, as the increased absorption by NO_2 is not sufficient to compensate for the decreased ozone absorption. In the case of the 1,000-fold enhancement, however, the increase in NO_2 absorption overwhelms the decrease in ozone, and the integrated solar radiation is decreased by several per cent at all latitudes.

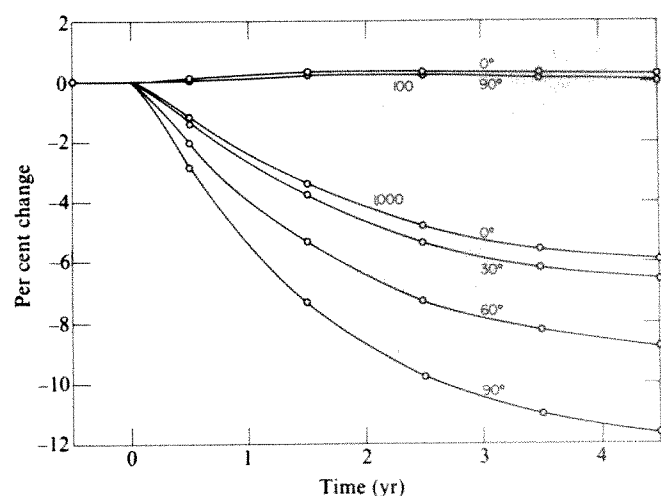


Fig. 5 Direct solar radiation penetrating the stratosphere integrated over one year and over all wavelengths.

In attempting to estimate the climatic effects of these changes in integrated solar radiation, it is important to realise that they are not equivalent to changes in the solar constant of the same magnitude. The radiation that is prevented from reaching the lower atmosphere in the 1,000-fold enhancement case is absorbed in the stratosphere, where it greatly increases the heating rate and thus the temperature, leading to a substantial increase in stratospheric thermal radiation. This partially compensates for the deficit in direct solar radiation at the surface. There will also be a change in the effective albedo of the lower atmosphere as a result of the change in spectral composition of the incident radiation.

These perturbations in the terrestrial radiation budget will certainly be accompanied by important changes in the dynamic coupling between the stratosphere and troposphere, and a calculation of the full climatic consequences is not yet possible. Estimates made with a relatively simple radiative-balance model, however, predict a drop in global average surface temperature of about 3 K for the 1,000-fold enhancement case. The complexities of radiative feedbacks are such that this figure is significant only as an indication of a major climatic perturbation, but is much larger than that estimated by Hunt¹¹, using a more sophisticated radiative-convective model. Hunt's calculation, however, included only the effects of the ozone depletion, and did not consider the much larger effect of the enhanced NO_2 that must accompany the depleted ozone.

Despite the qualitative nature of the estimated global cooling, some of the consequences are worth exploring. A 3 K drop in surface temperature, for example, would lead to a drop in global average atmospheric water-vapour content of about 20%, assuming a constant average tropospheric relative humidity profile as quoted by Manabe and Wetherald¹⁵. This would decrease the greenhouse effect, tending to decrease surface temperatures even more and would also lead to changes in cloud properties, with further climatic consequences. The reduction in water-vapour content might be expected to lead to widespread drought conditions, but might prevent the occurrence of a true 'ice age', as above-normal precipitation is probably required for the development of continental ice sheets.

Figure 6 shows the increase in the biologically effective UV radiation penetrating the stratosphere in the course of a year at the indicated latitudes for the two cases. These values were obtained by multiplying the integrated annual radiation at wavelengths between 280 and 310 nm by a 'biological effectiveness' factor¹⁶ derived on the basis of the absorption cross sections of protein and nucleic acids, and integrating over wavelength. Increases in UV dose by more than an order of magnitude are indicated, but these should be regarded with caution, as the effects of Rayleigh scattering by the lower atmosphere, which are substantial for UV wavelengths, have not been included. The results are thus only semi-qualitative, but indicate a severe increase in UV radiation levels.

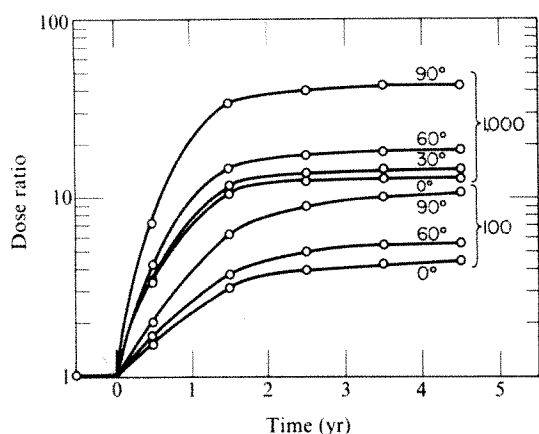


Fig. 6 Biologically effective annual UV dose during periods of enhanced cosmic-ray flux. Quantity shown is the ratio of the enhanced dose to that received in present conditions. Transmission through the lower atmosphere has not been included in either enhanced or normal dose.

The reduction in visible radiation intensity resulting from the 1,000-fold enhancement case is also likely to have an effect on global photosynthetic activity, as noted previously by Crutzen and Reid¹⁷. Photosynthesis is most efficient at wavelengths in the blue (near 450 nm) and in the red (near 660 nm), and Fig. 4a shows a reduction in the important blue light of about 30%. The integrated annual radiation in this wavelength region received at the Equator is thus equivalent to that received in present conditions at 40° latitude, and the radiation received at 40° is equivalent to that now received at 60°. Coupled with the probable drought conditions resulting from the decrease in global temperature, a severe decrease in the abundance of plant life is probable. According to our present understanding of the terrestrial carbon cycle, the decrease in plant life would probably lead to an increase in atmospheric CO₂ concentrations, which in turn would tend to increase the surface temperature through the greenhouse effect. The temperature might become stabilised in this way at a value somewhat above that predicted in the absence of the biosphere, but with a globally reduced biomass.

A further perturbation to the biosphere might arise from the increased precipitation of fixed nitrogen on the surface of the Earth. The ultimate fate of fixed nitrogen diffusing into the troposphere from above is probably rainout leading to surface deposition. The model calculations show that the total downward flux of N at tropopause heights is about $2 \times 10^{10} \text{ kg yr}^{-1}$ for the 100-fold enhancement case and $2 \times 10^{11} \text{ kg yr}^{-1}$ for the 1,000-fold enhancement case. The former value is comparable with current estimates for the annual amount of nitrogen fixed in the atmosphere by lightning¹⁸, and the latter value is comparable to the total amount of nitrogen fixed by biological processes¹⁹. The addition of such large quantities of biological nutrient would have an impact on the biosphere that is not easily estimated.

Conclusions

We may conclude that passage of the Earth through a SNR would be likely to cause a prolonged period of harsh environmental conditions for the biosphere, including high levels of damaging UV radiation, cooler global temperatures and generally dry conditions (depending on how intense the cosmic-ray flux within the SNR becomes), and reduced photosynthetic activity. Whether or not these severe conditions would lead to major extinctions of animal populations is a matter for speculation. The evidence from the geological and fossil record is not clear, although McLean²⁰ has interpreted the Cretaceous-Tertiary record as supporting a global warming, rather than a cooling, as the cause of the terminal Mesozoic extinctions. If this view is correct, a nearby supernova event can probably be eliminated as the cause of these particular extinctions.

The probability of a nearby (within about 15 pc) supernova explosion is also debatable. At the low end of the scale, Whitten *et al.*¹⁰ have estimated a 1–5% chance of a supernova within 5–10 pc in a period of 10^8 yr . Data presented by Shklovsky²¹ lead to an estimated frequency of one in $1.5 \times 10^8 \text{ yr}$ at 15 pc distance, while Clark *et al.*¹² estimate one in 10^8 yr at 10 pc distance on the basis of the confinement of type 2 supernovas to the vicinity of the spiral arms of the Galaxy, and the regularity of the passage of the Solar System through these spiral arms. Whichever view is correct, a nearby supernova explosion is not a dim and exotic possibility when viewed in the geological time scale, but rather an event that may well have occurred several times within the 10^9-yr lifetime of the biosphere.

Finally, note that enhancements in cosmic-ray flux by a factor of 1,000 lasting for 1,000 yr would cause an enhancement in the average cosmic-ray flux over a 10^8-yr period of only 1%. Such events would therefore be quite consistent with evidence from lunar rocks and meteorites that the average cosmic-ray flux has varied by less than a factor of two over the past 10^9 yr (ref. 22).

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The origin of labyrinth and tower karst and the climatic conditions necessary for their development

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A new karst style, labyrinth karst, is recognised in climates ranging from humid tropical to subarctic. In the late stages of evolution it is replaced by limestone towers indicating that tower karst is polygenetic, and not specific to the humid tropics as was once thought.

THE morphoclimatic principle that specific climates produce specific landforms has been more frequently applied in the study of karst (solutional) landscapes than in other branches of geomorphology. The impetus for its adoption came from pioneer studies of humid tropical karstlands in the 1930's¹. These revealed a wider variety of forms developed on a grander scale than elsewhere, and two broad karst styles—cone karst (Ger. Kegelkarst) and tower karst (Ger. Turmkarst)—that did not seem to develop in temperate or colder areas. In later work climate has been called upon to explain both areal and altitudinal variations in the character of karstlands. Despite reaction to what some consider as a grave overemphasis of climatic factors^{2,3}, the principle is still widely accepted. Central to that acceptance is the supposed humid tropical climaspecificity of the cone and tower karst styles, for which thresholds of temperature and precipitation have been proposed (Table 1).

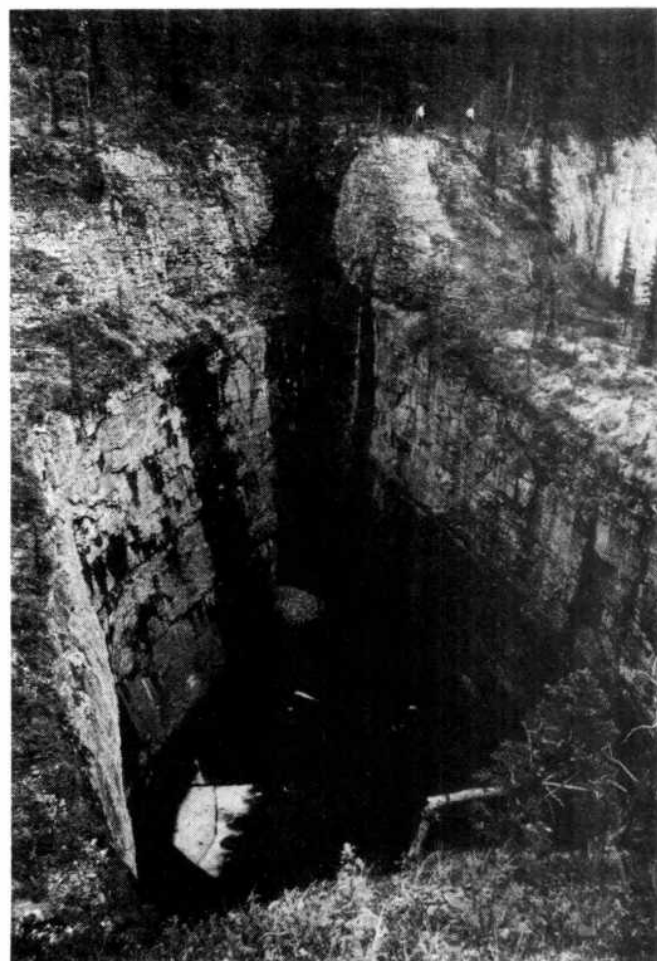
In 1972–74 we explored and studied a complex and accentuated karstland at lat 61–62° N in the Nahanni area of the Mackenzie Mountains, Northwest Territories, Canada. Landforms are at an altitude of 1,000–1,300 m. The climate is of the subarctic continental interior type and there is discontinuous permafrost. Our findings have added to knowledge of karst styles and suggest that some relationships between karst morphology and climate be reassessed.

The Nahanni karst is dominated by a series of vertical-walled landforms which are linked in a simple yet remarkable developmental sequence^{12,13}. During early stages water draining underground via vertical fissures creates strings of solution dolines, circular to elliptical in plan view and cylindrical in depth (Fig. 1). By enlargement and coalescence along the fractures, strings of dolines are converted to intersecting networks of karst streets. As streets deepen and widen, the intervening rock ridges are dissected and ultimately destroyed. Replacing them are large closed depressions of angular planform and irregular distribution that we term 'karst platea'. Where insoluble sediment is carried to these depressions by allogenic streams, underground drainage becomes impeded and there is flooding after heavy summer rains. Floors are alluviated and the platea become small poljes. As platea and/or poljes expand, rock towers are left which rise from uneven karst margin plains. At this stage of evolution the landscape resembles a scaled-down version of the spectacular tower karsts of Puerto Rico or south

China. In the north of the Nahanni region karst streets, platea, and poljes form an intricate natural rock labyrinth (Fig. 2). Streets are as much as 185 m deep and 9 km long, and platea and poljes up to 125 m deep and 2–3 km long. Limestone towers with vertical to overhanging walls are up to 125 m high (Fig. 3).

We have identified karst landscapes like that of the Nahanni in several humid and seasonally humid tropical carbonate terrains. In south-west Celebes, Sunartadirdja and Lehmann¹⁴ describe

Fig. 1 The early doline stage of labyrinth karst development. Rappel Cenote is an elliptical-shaped doline 30 m deep. Even in summer drainage routes are blocked by subsurface ice so that ponds are common in such depressions.



karst streets 6–400 m wide, 50–200 m deep, and up to 8 km long. Where these intersect, depressions (platea) have developed. Several have been alluviated by influx of insoluble residue from nearby crystalline rock indicating that they are the embryos of future poljes. Scattered, isolated limestone towers are also common. Similar topography is found on many upland surfaces in Sarawak¹⁵. For example, at Subis joint-guided corridors (streets) are about 30 m apart and 3–150 m deep. At intersections depressions (platea) 5–180 m deep and 5–500 m across are common. At an advanced stage of dissection rows of tall 'needles' (towers) are all that remain between the streets. Stereo photographs (Plate 2 of ref. 15) reveal a rock labyrinth nearly identical to that in the Nahanni. Deep, intersecting karst streets several kilometres long are reported in the Star Mountains and Lake Ajamaru regions of New Guinea^{2,16}. They are also clearly visible in oblique photographs of the Sierra Maestra in Cuba (Fig. 16 of ref. 3), and of the 'Ruined City' of Arnhem Land, Northern Territory, Australia (Fig. XIV-24 of ref. 17) where they isolate numerous pinnacles, towers, and ridges. Less spectacular labyrinths are known in the Tanga region of Tanzania where streets and towers have a maximum relief of 15 m (ref. 18), and in Yunnan, south China, where corridors with ridges and pinnacles up to 30 m high form a landscape that has been described as a "tropical rock forest"¹⁹.

Natural rock labyrinths and associated tower karst are also described in tropical semiarid areas. In the Limestone Ranges of the Fitzroy Basin, Western Australia²⁰, intersecting networks of karst streets up to 6 m wide, 30 m deep, and hundreds of metres long form large areas of 'dissected karst' which include enclosed depressions of angular planform up to 1.5 km long, 180 m wide,

Fig. 2 Aerial photograph of the Nahanni showing labyrinth karst terrain with streets, platea, poljes, and isolated towers.

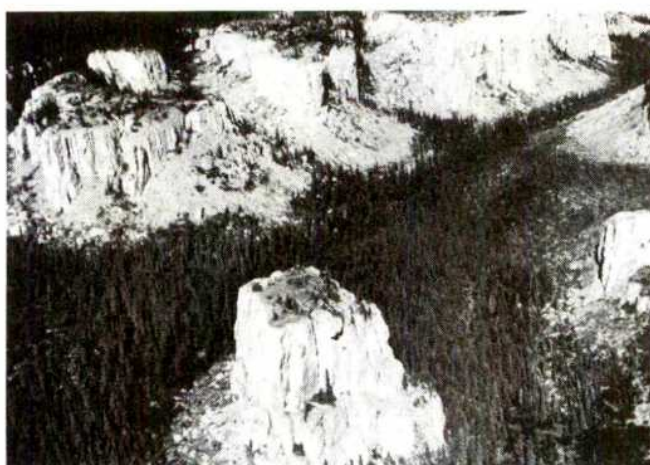
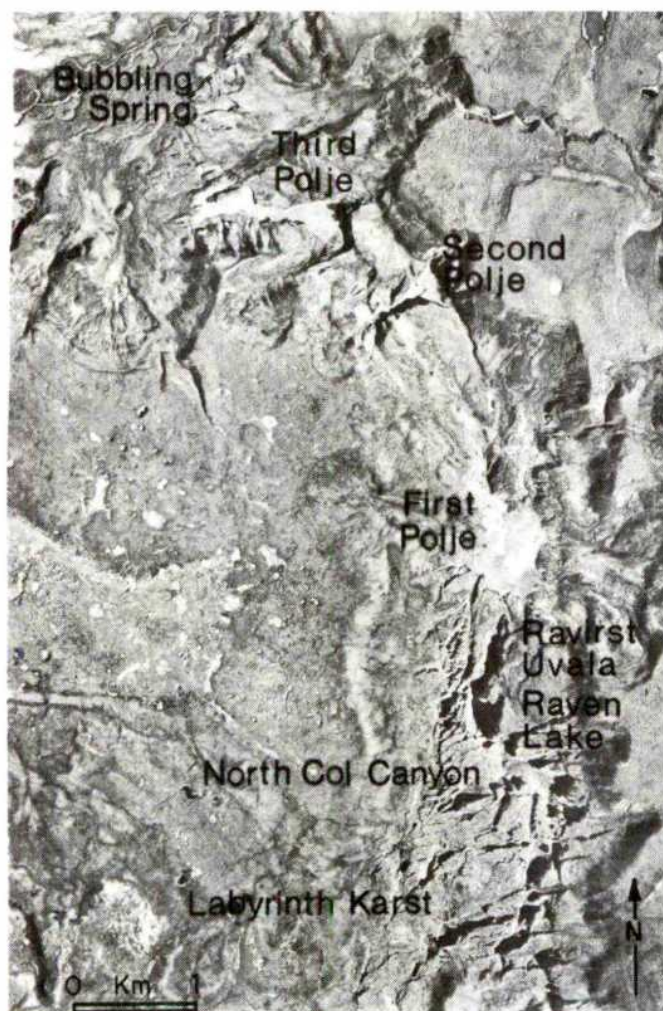


Fig. 3 A late tower karst stage in the evolution of labyrinth karst in the Nahanni. Insel Tower is approximately 65 m high and rises from a flat alluviated plain.

and 30–45 m deep. These have steep to vertical walls, and flat floors almost entirely mantled by alluvium or residual soil. Jennings and Sweeting²⁰ refer to them as "miniature poljes" but they may well be the semiarid equivalents of karst platea. In addition to the dissected karst are areas of towers, pinnacles, and narrow ridges up to 45 m high which rise from a rock pediplain. The karst of Bom Jesus da Lapa, Brazil, in a limestone hill 2 × 1 km and 200 m high, is similar to the Fitzroy terrain²¹. The hill is crossed by street networks 1–2 m wide and 10–30 m deep which isolate limestone pinnacles, ridges, and towers. Also common are circular solution pits 2–5 m in diameter and 10–30 m deep which appear identical to the dolines that form during the early stage of karstification at Nahanni.

The existence of such similar rock labyrinths in widely distributed carbonate terrains, and of shallower street networks elsewhere^{22–29}, demonstrates that a new karst style—"labyrinth karst"—must be recognised. This style can develop at a variety of scales, with individual depressions ranging from several centimetres to a few hundred metres in depth¹³. During the later stages of evolution a tower karst landscape emerges either directly from karst street networks, or through intermediate platea or platea and polje stages. Variations in morphology between labyrinths are slight and reflect differences in lithology, climate, and in the availability of insoluble residue. The climatic effect is minor. For example, in the Nahanni karst, depression floors and walls are frequently mantled by accumulations of frost-shattered limestone while in the hot semiarid Fitzroy Basin of Australia, where pedimentation processes influence karst evolution, rock debris is uncommon.

In many tropical karstlands two successive stages of erosion can be recognised^{4,5,10}. Cone karst develops until the floors of cockpits extend to local base levels and significant quantities of alluvium are retained. Thresholds between adjacent depressions are submerged, and cones are left standing above broadening alluvial plains. Lateral solutional undercutting may then transform the cones into steep-sided limestone towers. Because true tower karst also forms during labyrinth karst evolution, the tower karst style is polygenetic³. It forms from cone karst when solution is concentrated in a single horizontal plane and from labyrinth karst because solution is concentrated in discrete vertical planes. In the one case tower karst is a conical karst formed under special hydrological, structural and lithological conditions^{6,7,30}, in the other it is entirely a product of particular structural and lithological characteristics.

In the Lake Ajamaru region of New Guinea Pannekoek¹⁶ has identified all stages in a karst cycle of erosion that commences with the formation of small surface valleys aligned along major joints. As water begins to drain underground joints are widened and clefts (karst streets) are opened, isolating angular limestone hills. At this stage the landscape conforms to labyrinth karst. As

Table 1 Suggested temperature and precipitation thresholds for cone and tower karst development

Source	Temperature threshold (°C)	Precipitation threshold (mm)	Area of applicability
Sweeting ⁴		1,840*	Jamaica
Gerstenhauer ⁵	high	1,000	Worldwide
Verstappen ⁶	17–20*	1,500	Worldwide
Balázs ⁷	18	1,200	Worldwide
Sweeting ⁸	18*	1,480*	South China
Williams ⁹	13*	3,250*	New Guinea
Jakucs ¹⁰	17–18	1,000–1,200	Worldwide
Monroe ¹¹		1,300*	Puerto Rico

* Values estimated from statements made by the various authors or from information given by them.

the clefts continue to widen the hills become smaller and lose their angularity so that Kegelkarst topography is produced. Karst on the northern flank of the Sierra Maestra, Cuba (Fig. 16 of ref. 3) seems to fit well into Pannekoek's cycle; residual towers and ridges within a labyrinth are becoming rounded and a landscape resembling oriented cone karst (Ger. gerichteter karst) is emerging. The indication is therefore, that like tower karst, cone karst is polygenetic and that in some areas labyrinth karst may be an early stage in its development.

Once the basic characteristics of cone and tower karst became known in the 1930's and 1940's, examples were reported well outside of modern humid and seasonally humid tropical limits^{31–34}. In almost every case these landscapes were interpreted as fossil, survivals from former periods of tropical climate. For example, Tricart and Da Silva²¹ maintain that the Bom Jesus da Lapa karst was fashioned in a humid Tertiary climate and that it has suffered only minor modification in the much drier conditions that have existed since the late Miocene. A similar interpretation has been put forward for mogotes in the Cracow Upland of Poland³¹, which are identical to the towers of Nahanni, and which are scattered over a similar-sized area (15×5 km). A fossil or palaeoclimatic interpretation of the Nahanni karst is untenable. Evidence such as the presence of abundant erratics from the Canadian Shield indicates that the area was glaciated more than once during the Pleistocene. The present karst landforms are developed from a glacially scoured limestone surface. They post-date the last Laurentide ice to cover the area. The karst therefore seems to have originated in climatic conditions that, on average, were at least as harsh as those of today—that is a mean annual temperature of –4.5°C and a precipitation of only 566 mm. These are well below the minimum conditions supposed necessary for tower karst development (Table 1).

Recent palaeogeographical work in Australia also shows that labyrinth and tower karst can form in cold climatic conditions. In the partly glaciogenic Lower Permian sequence of the Carnarvon Basin, Western Australia is a locally-exhumed karst landscape covering approximately 1,000 km². Karst streets up to 20 m wide, and pinnacle-shaped towers up to 30 m high are developed in calcarenite of the Callytharra Formation. A thick tillite-rich sequence underlies the Callytharra and the karst is overlain by marine sediments containing rare boulder-size erratics. In addition, all Lower Permian faunas from this region indicate cool to temperate conditions (van de Graaff, W. J. E. Hocking, R. M. and Denman, P. D., Geological Survey of Western Australia, personal communication).

Recognition of labyrinth and associated tower karst in climates ranging from humid tropical to dry subarctic suggests that, like doline karst, these styles are not primarily climate or latitude dependent (Table 2). Why then do deep labyrinths and isolated towers develop in some areas and not others, and why are they more common in the tropics? Two local factors seem to have been important in the formation of the Nahanni labyrinths. First, this area was beyond the limits of the Wisconsinan (last) Laurentide ice sheet, and may even have escaped glaciation during the Illinoian period³⁶. It is one of few high-latitude carbonate regions where karst has had time to develop fully, without drastic interruption by flowing ice. Second, although mean soil P_{CO_2} (0.3%), solution intensity ($CaCO_3$ 144 mg l⁻¹), and solute denudation (17.9–27.1 m³ km⁻² yr⁻¹), are much lower than in the tropics, (where the average soil P_{CO_2} at 30 cm depth is 2.0%³⁷, mean water hardness lies between 165 and 180 mg l⁻¹ $CaCO_3$ ^{37,38} and estimated denudation rates include 83 m³ km⁻² yr⁻¹ for Indonesia³⁹, 50–60 m³ km⁻² yr⁻¹ for the Philippines⁴⁰, and 72 m³ km⁻² yr⁻¹ for Jamaica⁴¹) solutational activity is not constant across the area. It seems significant that the deepest solutational landforms are located in a zone that receives large volumes of acid allogenic water from nearby shales, and that elsewhere only shallow karst street networks are encountered. Annual denudation in this zone is two to three times the regional average bringing it close to rates typical of tropical cone and tower regions. At the present rate—approximately 50 m³ km⁻² yr⁻¹—it is estimated that the Nahanni landscape could have formed within the last 200,000 yr, a period that has been available for uninterrupted karst development.

The local factors explain why the Nahanni karst is more highly developed than other subarctic carbonate terrains but not why labyrinth and tower karst predominate, rather than other styles such as doline karst. Many temperate karstlands have longer uninterrupted histories of evolution than the Nahanni, and display greater solute concentrations, runoff and denudation rates than some humid tropical regions³⁷. Yet they lack towers and networks of deep karst streets. Total denudation, therefore, does not determine labyrinth karst development; it seems that structural and lithological properties of the host rock are

Table 2 Areas and climates in which deep labyrinths and late-stage tower karst have been discovered*

Area	Ref.	Approximate latitude	Köppen climate	Temperature (°C)	Precipitation (mm)
Maros, Celebes	14	5°S	Af	26.4	2,850–3,360
Lake Ajamaru, New Guinea	16	2–4°S	Af	26.6–28.3	2,490–2,845
Star Mountains, New Guinea	2	3–4°S	Af		
Arnhem Land, Australia	17	12–14°S	Aw	26.6	1,070
Sierra Maestra, Cuba	3	20°N	Aw	25.3	1,090
Tanga, Tanzania	18	5°S	Aw	26.0	1,350
Bom Jesus da Lapa, Brazil	21	9–11°S	BShw	26.5	700–900
Fitzroy Basin, Australia	20	17–19°S	BShw	27.6	460–710
Carnarvon Basin, Australia	†	50–60°S‡		Temperate or cold	566
Nahanni, Canada	12	61–62°N	Dcf		

* Information given by the authors listed was supplemented from a variety of other sources.
† van de Graaff *et al.*, personal communication (see text).
‡ The position of the Carnarvon area during the early Permian period was estimated from diagrams contained in ref. 35.

primarily responsible. Deep labyrinths are restricted to massive carbonates of low porosity and primary permeability that are crossed by widely spaced, but vertically and horizontally extensive, fractures or fracture zones. There are very few penetrable bedding planes. In many cases the fault and joint networks are results of recent uplift and flexing, which also provided the necessary local relief. Limestones and dolomites suitable for deep labyrinth karst development are comparatively uncommon explaining why this style is not more frequently encountered. Solutional labyrinths of much smaller scale, confined to the uppermost few metres of rock, are more numerous and are termed 'karrenfeld' or 'limestone pavements'^{42,43}.

Although certain broad influences of zonal climate on karst morphology cannot be denied, characteristics of the host carbonate shape the labyrinth karst style. Given an equal distribution of suitable rocks it is to be expected that there will be a greater frequency of this style in the tropics where karst evolution has not been interrupted by glacial events. Tower karst can no longer be regarded as specific to humid tropical climates for it can develop well in cold environments. Some cases where extra-tropical tower karst has been interpreted in terms of tropical palaeoclimatic conditions may require re-evaluation. If the distribution of tower karst is not dependent on climate one of two fundamental pieces of evidence that uphold the morphoclimatic approach to karst geomorphology is no longer valid. In future, more emphasis should be given to structural and lithological properties of the host soluble rock.

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Temperature-sensitive mutant of avian erythroblastosis virus suggests a block of differentiation as mechanism of leukaemogenesis

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A temperature sensitive mutant has been isolated for the first time from a replication defective acute leukaemia virus, AEV. In vivo, at 41 °C, the mutant shows a reduced leukaemogenic potential. In vitro, in erythroblasts transformed at 35 °C, haemoglobin synthesis can be induced by a shift to 41 °C. This indicates that the continuous expression of a viral gene product is necessary to maintain the undifferentiated state of the virus-transformed leukaemia cells.

THE development of *in vitro* transformation techniques for replication-defective leukaemia viruses in cultures of haematopoietic cells (see ref. 1 for review) introduced a new approach for the study of the mechanism of virus-induced leukaemogenesis. Results obtained mainly with avian erythroblastosis virus (AEV), myeloblastosis virus (AMV) and myelocytomatosis

virus strain MC29 and with the murine Friend and Abelson leukaemia viruses showed that these viruses selectively transform haematopoietic cells of different lineages of differentiation in a highly selective manner¹. Two important questions were raised by these studies. First, is the leukaemic transformation effected by these viruses accompanied by a block of differentiation in their target cells? And second, is a viral gene product necessary to maintain the leukaemic state of the infected cell?

To answer these questions we decided to search for temperature-sensitive mutants of AEV. This virus induces an acute erythroblastosis within 1–2 weeks post-infection if injected intravenously, and slowly developing sarcomas if injected intramuscularly². After infection of bone marrow cells in culture it induces the formation of rapidly proliferating erythroblast foci or colonies^{3–5}. It also induces a transformation of chicken embryo fibroblast cultures^{4,6}. However, AEV does not possess the *src* gene, the gene responsible for transformation of fibroblasts by RSV⁷.

This article reports the isolation of the first temperature-sensitive mutant of AEV and partially answers the questions posed above.

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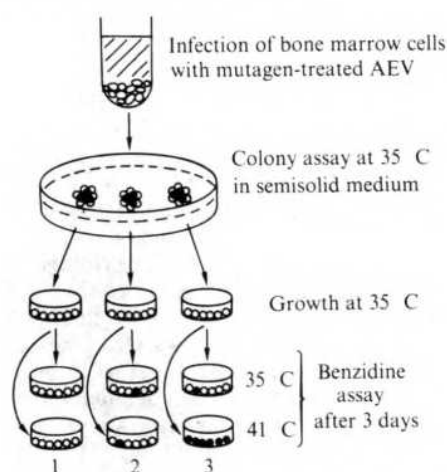


Fig. 1 Schematic representation of the procedure used to isolate temperature-sensitive mutants of AEV. The AEV used was a RAV-2 pseudotype of AEV-ES4 obtained as described earlier⁴. The virus was treated by mutagen by adding $5 \mu\text{g ml}^{-1}$ 5-azacytidine (Calbiochem) to 45×10^6 erythroblasts producing AEV in 10 ml of growth medium⁴. After 18 h at 37°C the erythroblasts were removed by low-speed centrifugation and the supernatant centrifuged at $70,000g$ for 30 min to pellet the virus. The pellet suspended in 5 ml growth medium was used to infect fresh bone marrow cells which were seeded at 10×10^6 cells per 35-mm dish in methylcellulose-containing medium (ref. 5, and in preparation). After 8–12 days of incubation at 35°C erythroblast colonies were isolated with a drawn out Pasteur pipette, transferred into the wells (15 mm) of a multidose tissue culture tray each containing 0.5 ml growth medium and propagated at 35°C until 10^5 – 10^6 cells were obtained from each colony. The erythroblast colonies were then suspended in 1 ml growth medium, and divided equally into the respective wells of two new trays. One tray was shifted to 41°C , the other was kept at 35°C . After 3 days of incubation at the respective temperatures all cultures were stained with benzidine by the procedure of Orkin *et al.*⁸ to determine the proportion of haemoglobin-positive (B^+) cells. In the scheme, B^+ cells are drawn as closed circles, B^- cells as open circles. In the example shown, clone 3 behaves as expected from an erythroblast clone infected with a temperature-sensitive mutant, that is, B^- at 35°C and B^+ at 41°C ; clones 1 and 2 behave like wild-type-infected cells.

Isolation of erythroblast clones with temperature-sensitive properties

Initial attempts to isolate *ts* mutants of AEV using the fibroblast focus assay⁴ failed to yield the desired mutants (B. Royer-Pokora and T.G., unpublished). With the development of a new transformation assay of bone marrow cells which allows the isolation of clones of transformed cells characterised as immature erythroid cells (erythroblasts) by various differentiation markers and derived from the infection with a single AEV particle (ref. 5, and H.B. *et al.*, in preparation), an alternative approach became possible. The analysis of a large number of individual erythroblast clones obtained with this technique revealed that different clones vary in their haemoglobin expression measured as the proportion of benzidine-positive (B^+) cells. Most of these clones contained less than 10% B^+ cells⁵; this proportion, however, could be greatly increased by the addition of butyric acid⁵. These and other findings not reported here led to the hypothesis that AEV induces a block in differentiation in its haematopoietic target cells. Consequently, if it were possible to inactivate the viral functions in the infected cell, it should be possible to induce a differentiation in the AEV-transformed erythroblasts. Based on this idea a strategy was developed for isolating temperature-sensitive mutants of AEV which is outlined in Fig. 1. Erythroblast colonies were produced by infecting bone marrow cultures with mutagen-treated AEV at 35°C and seeding them into semisolid medium. Cells obtained from individual colonies were propagated at

35°C . Aliquots of these clones were then shifted to 41°C or kept at 35°C and assayed for the proportion of B^+ cells by benzidine staining 3 days later.

From 94 erythroblast clones derived from mutagen-treated virus and assayed in this way, three clones (numbers 3, 34 and 43) were temperature-sensitive for haemoglobin expression, containing more than 70% strongly B^+ cells at 41°C as compared to <10% at 35°C . In contrast, none of 26 colonies derived from untreated AEV showed this type of temperature sensitivity.

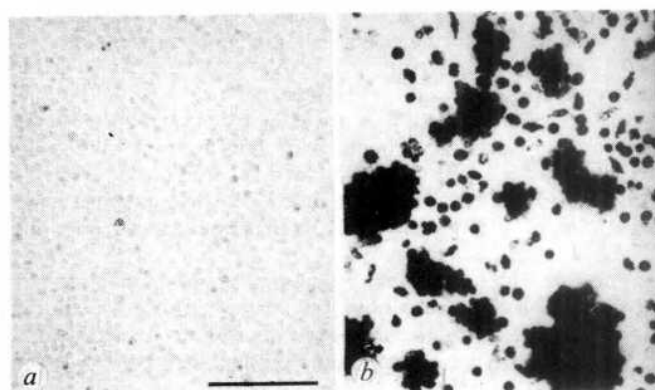
AEV from erythroblast clone 34 is temperature sensitive

To determine whether clones 34 and 43 produce virus capable of inducing erythroblast colonies temperature sensitive for haemoglobin expression (clone 3 was lost), cell-free supernatants from both clones were used to infect fresh bone marrow cultures at 35°C . In addition, bone marrow cells were infected with wild type (*wt*) AEV. Between 300 and 1,000 colonies per dish were obtained with the three inocula. Twenty colonies of each type were isolated at random and the 10 fastest growing clones were tested for temperature sensitivity. All of the 10 erythroblast clones derived from clone 34 virus (*ts*34 AEV) could be induced to express haemoglobin, showing an increase from an average of about 13% B^+ cells at 35°C to about 76% at 41°C (Table 1, expt 1). In contrast, with the exception of clone 4, *wt* AEV-infected clones showed essentially identical, relatively low proportions of B^+ cells at both 35 and 41°C (Table 1, expt 1). The virus produced by clone 4 was characterised as *wt* AEV by its inability to induce temperature-sensitive erythroblast colonies (data not shown). The 10 clones induced by virus from clone 43 cells exhibited variable proportions of B^+ cells at 41°C , fluctuating between less than 1% to 59% or greater with an average of 32% (data not shown). It is remarkable that with *ts*34 AEV, in general, those clones which already exhibited relatively high proportions of B^+ cells at 35°C also showed the highest proportions of B^+ cells after shift to 41°C . Photographs of *ts*34 AEV-infected erythroblasts kept at 35°C and at 41°C and stained with benzidine are shown in Fig. 2.

The *ts* lesion is located in the defective, transforming component of *ts*34 AEV

The above results indicate that the temperature sensitivity of haemoglobin expression in erythroblast clones 34 and possibly 43 was due to the infecting AEV/helper virus complex and not to a property inherent to the particular host cells. To determine whether the temperature sensitivity of *ts*34 AEV was due to a mutation in AEV rather than in the helper virus (RAV-2),

Fig. 2 Effect of temperature on the expression of haemoglobin in *ts*34 AEV-infected erythroblasts. Erythroblasts from a single clone were grown at 35°C and aliquots seeded at 35°C (a) and 41°C (b). Three days later the cells were stained with benzidine. The dark cells represent B^+ cells. Aggregation of cells at 41°C usually precedes the appearance of B^+ cells by 1–2 d. Scale bar, 100 μm .



attempts were made to obtain nonproducer erythroblasts containing only the AEV genome. Erythroblast colonies were isolated from bone marrow suspension cultures infected with *ts*34 AEV at low multiplicity. After propagation of the isolated colonies at 35 °C, the supernatants were assayed for reverse transcriptase activity (AEV-transformed nonproducer erythroblasts do not release reverse transcriptase-positive virus particles⁹) and for the production of infectious virus using the bone marrow colony assay to score for nonproducer erythroblast clones. Of eight clones tested, four were found to be nonproducer by both types of assay. All eight *ts*34 AEV-infected erythroblast clones, regardless of whether or not they produced virus, were temperature sensitive for the expression of haemoglobin. This demonstrates that a lesion in AEV and not in the helper virus is responsible for the observed temperature-sensitive expression of haemoglobin in *ts*34 AEV-infected cells. To further substantiate this conclusion, pseudotypes of *ts*34 AEV were produced by superinfection of a *ts*34 AEV nonproducer clone with standard helper viruses. Colonies induced by these pseudotypes were then tested for temperature

Quantitation of haemoglobin expression in *ts*34 AEV-infected erythroblasts

Two types of assay were used to obtain quantitative estimates of haemoglobin expression after shift of *ts*34 AEV- or *wt* AEV-infected erythroblast clones to 41 °C. First, cell extracts were reacted with benzidine and assayed photometrically; and second, a newly developed competition radioimmunoassay for chicken haemoglobin was used. For comparison, before extraction the same clones were subjected to the cytological benzidine assay. The results of these experiments are shown in Table 2. With the benzidine photometric assay a 10- to 15-fold increase was observed in the *ts*-clones 3 days after shift to 41 °C as compared to a 2 to 4-fold increase in the *wt* clones. When the same extracts were tested for haemoglobin by competition radioimmunoassay, a 5 to 7-fold increase was observed in the *ts* clones whereas no or only a slight increase (1.5–2.5-fold) was observed in the *wt* clones.

The smaller increase in haemoglobin levels of the *ts* cells observed by these techniques as compared to the cytological

Table 1 Assay of temperature sensitivity of erythroblasts infected with various pseudotypes of *ts*34 AEV and *wt* AEV

Expt	Virus used for infection	Incubation temperature (°C)	Clone no. (% of B ⁺ cells)									
			1	2	3	4	5	6	7	8	9	10
Expt 1	<i>ts</i> 34 AEV	41°	65	59	59	60	98	91	91	95	61	87
		35°	0	1	4	2	33	33	17	37	2	18
	<i>wt</i> AEV	41°	0	0	0	49	0	16	0	0	13	0
		35°	0	0	0	26	0	31	0	0	13	0
Expt 2	<i>ts</i> 34 AEV (RAV-2)	41°	35	60	55	40	90	75	25	60	—	—
		35°	1	1	4	1	22	5	0	4	—	—
	<i>ts</i> 34 AEV (SR-RSV-D)	41°	55	75	—	—	—	—	—	—	—	—
		35°	5	8	—	—	—	—	—	—	—	—
	<i>ts</i> 34 AEV(<i>wt</i> AEV)	41°	85	55	0	60	1	2	60	—	—	—
		35°	16	17	0	0	0	0	1	—	—	—
	<i>wt</i> AEV(RAV-2 <i>ts</i> 34)	41°	0	1	1	25	0	0	5	2	—	—
		35°	0	1	0	17	0	0	2	0	—	—

Pseudotypes of *ts*34 AEV were obtained by inoculating 5 × 10⁵ cells of a clone of *ts*34 AEV nonproducer erythroblasts at 35 °C with about 10⁵ infectious units of RAV-2, SR-RSV-D or AEV (RAV-2). The *wt* AEV (RAV-2 *ts*34) pseudotype was obtained by superinfecting a clone of *wt* AEV nonproducer erythroblasts⁹ with RAV-2 which had been plaque purified¹⁰ from the original stock of *ts*34 AEV. Virus collected 5 days post-infection (p.i.) was used to infect bone marrow cultures at 35 °C. Twenty to 80 colonies were obtained in the cultures infected with the pseudotypes, no colonies were obtained with the supernatant of nonproducer erythroblasts without added helper virus. Colonies were isolated, propagated and tested as described in Fig. 1. In the cultures stained with benzidine a minimum of 250 cells were evaluated. In expt 1, erythroblasts were shifted 17 days p.i. (that is, at about 15–20 population doublings), in expt 2, 24 days p.i. (~22–27 population doublings).

sensitivity. Regardless of whether RAV-2, SR-RSV-D (Table 1, expt 2) or RAV-1 (data not shown) were used as helper virus, all pseudotypes of *ts*34 AEV still induced temperature-sensitive erythroblast colonies. In a similar experiment using *wt* AEV (which contains RAV-2 as helper virus) for superinfection of *ts*34 AEV nonproducer erythroblasts, the resulting virus mixture induced both wild-type (3 out of 7) and temperature-sensitive (4 out of 7) colonies (Table 1, expt 2). This indicates that *ts*34 AEV-infected erythroblasts can be superinfected with *wt* AEV.

An independent line of evidence shows that the original helper virus of *ts*34 AEV is not responsible for the observed temperature-sensitive properties of the latter: a pseudotype consisting of *wt* AEV and the helper virus isolated from the original *ts*34 AEV virus stock failed to cause colonies which were temperature sensitive for the expression of haemoglobin (Table 1, expt 2).

These results suggest that a gene product of AEV blocks the accumulation of haemoglobin, one of the major events during normal erythroid differentiation. In the following, this notion is substantiated by quantitative data on haemoglobin expression and by kinetic studies.

assay probably reflects a threshold effect of the latter method. The expression of haemoglobin in the induced erythroblasts is still far less complete than in fully differentiated cells: depending on technique used, only 2 to 8% of the haemoglobin levels of erythrocytes is expressed in the *ts*34 AEV erythroblasts 3 days after shift. Assuming that the benzidine technique measures the levels of protein-bound and free haem and that the radioimmunoassay detects the levels of haemoglobin as well as free globin, the data in Table 2 suggest that there is a more pronounced or more rapid induction of haem synthesis as compared to globin synthesis in these cells in response to the shift. In addition, our data indicate that in AEV-infected erythroblasts at the 'undifferentiated' stage there is an excess of globin over haem, assuming that equimolar amounts of haem and globin are present in mature erythrocytes.

Kinetics and reversibility of haemoglobin expression

To study the time course of temperature-dependent induction of haemoglobin synthesis, *ts*34 AEV-infected erythroblasts were shifted from 35 to 41 °C and aliquots were stained with ben-

Table 2 Haemoglobin expression in *ts34* AEV-infected erythroblasts

Cells tested		% Benzidine positive cells	Hb expression in cell extracts:		
			Benzidine staining	Competition radioimmunoassay	
			A_{515} per mg protein	μ g haemoglobin per mg protein	
			41°/35°	41°/35°	
<i>ts34</i> AEV-erythroblasts	35 °C	0, 1.4, 1.7	0.8, 1.0, 0.8	5.4, 5.4, 6.2	
	41 °C	73, 68, 87	8.2, 9.2, 10.6	38, 32, 32	7.1, 5.8, 5.1
<i>wt</i> AEV-erythroblasts	35 °C	0, 2.4, 1.6	<0.2, 1.2, 0.6	3.8, 16.6, 4.4	
	41 °C	0.1, 7.2, 3.9	1.6, 2.2, 2.0	7.2, 12.4, 12.8	1.9, 0.8, 2.8
Chicken erythrocytes		100, 100	446, 394	474, 528	—
Chicken fibroblasts, macrophages		0, 0	<0.2, <0.2	<0.5, <0.5	—

Three clones each of *ts34* AEV- and *wt* AEV-‘transformed’ erythroblasts grown at 35 °C for ~25 population doublings were seeded at 12×10^6 cells in 10 ml growth medium in two 100-mm dishes. One of the dishes was kept at 35 °C, the other was shifted to 41 °C. After 3 days, small aliquots of each clone were separated for protein determination¹² and for staining with benzidine⁸ to reveal the proportion of B⁺ cells and with Trypan blue to reveal the proportion of dead cells. Cell extracts were prepared essentially according to the procedure of Conscience *et al.*¹¹. Of these extracts, 5–50 μ l were used for benzidine staining using the procedure of Crosby *et al.*¹³ and for the competition radioimmunoassay as outlined below. The values shown are the data obtained with the three different clones whereby position 1 always corresponds to clone 1, position 2 to clone 2 and position 3 to clone 3. The values shown were corrected for the proportion of dead cells. Competition radioimmunoassay: chicken haemoglobin was prepared from washed erythrocytes of adult chickens as described by Bruns and Ingram¹⁴. This preparation (~90% pure by SDS-polyacrylamide gel electrophoresis and staining with Coomassie blue) was concentrated to 11 mg ml⁻¹ by ultrafiltration and dialysed against 0.1 M borate buffer pH 8.5. Haemoglobin (30–60 μ g) was then labelled with 0.5–1 mCi [¹²⁵I]Bolton-Hunter reagent (Amersham, 1,700 Ci mmol⁻¹) as described¹⁵. Labelled protein was separated from unreacted iodinated ester and other by-products on a Sephadex G75 superfine (Pharmacia) column (0.5 $\times$ 10 cm) equilibrated with 0.1 M borate buffer pH 8.5 containing 0.25% gelatine. After the addition of 1% Triton X-100 the labelled haemoglobin was stored in aliquots at -70 °C for up to 6 weeks. Radioactivity of the labelled product was between 3 and 6 $\times 10^6$ c.p.m. per μ g protein and of this >90% was in haemoglobin as judged by SDS-polyacrylamide gel electrophoresis and autoradiography. More than 60% of the labelled product was precipitable with an excess of antibody. The reaction mixture for competition radioimmunoassay contained between 50,000 and 60,000 c.p.m. (± 10 –20 ng) of [¹²⁵I]-haemoglobin, 2.5 μ g of rabbit immunoglobulin directed against chicken haemoglobin (Cappel Laboratories) dissolved in 10 μ l RIA-buffer (0.01 M Tris pH 7.0; 0.1 M NaCl and 1% Triton X-100), 5–50 μ l cell extract to be assayed for haemoglobin and RIA-buffer up to a final volume of 400 μ l. After incubation for 2 h at 37 °C and 2 h at 4 °C, 10 μ l of a 10% (v/v) *Staphylococcus aureus* suspension prepared according to Kessler¹⁶ were added and the mixture incubated for another 30 min at 37 °C. The reaction mixture was washed by centrifugation through 500 μ l of 10% sucrose in RIA buffer and the pellet was resuspended in 600 μ l RIA buffer followed by another centrifugation. The radioactivity of the sediment was then determined in a gamma counter. In a typical calibration curve, using a standard haemoglobin preparation, 2 ng of cold haemoglobin resulted in 20% competition; 9 ng in 50% competition and 60 ng in 90% competition.

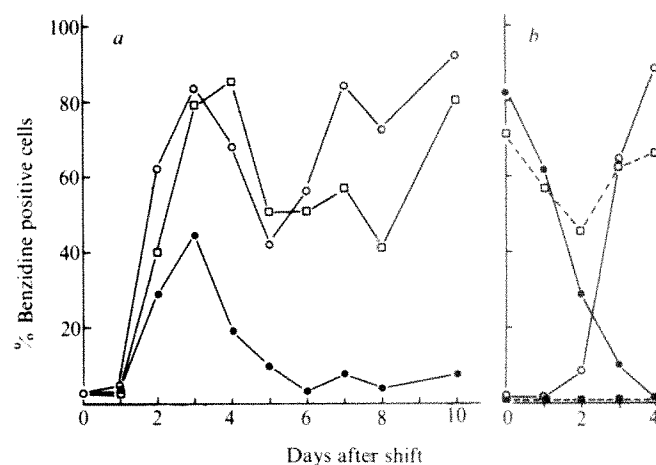
zidine at daily intervals thereafter. As can be seen from Fig. 3, the proportion of B⁺ cells reached a maximum 3 to 4 days after shift. Thereafter, in most experiments, a slight temporary decline was seen which remains unexplained so far. No significant difference in the kinetics was observed in the absence or presence of helper virus (Fig. 3a). After superinfection of *ts34* AEV nonproducer erythroblasts with *wt* AEV at the time of shift to 41 °C, only a transient expression of haemoglobin within the first 2 days was observed. After 4 to 6 days, haemoglobin expression was again suppressed almost to control levels (Fig. 3a). As shown in Fig. 3b, the increased expression of haemoglobin induced by shift to 41 °C could also be reversed by a subsequent downshift of the *ts34* AEV erythroblasts to 35 °C, exhibiting a similar time course as seen in the upshift experiments.

Leukaemogenicity of *ts34* AEV

If *ts34* AEV does in fact cause a block of differentiation in its target cells at 35 but not at 41 °C, and if this differentiation block is correlated to or even causative of leukaemic transformation, then *ts34* AEV should also exhibit a decreased *in vitro* transforming ability at 41 °C as well as a reduced leukaemogenicity *in vivo* (the body temperature of the chicken exceeds 41 °C).

Assay of *ts34* AEV in bone marrow cells using the methylcellulose colony assay yielded comparable numbers of colonies at 35 and 37 °C (580 and 480) but no colonies at 39 °C. In contrast, however, the number of colonies obtained with *wt* AEV at 39 °C (330) was only slightly lower than at 35 and 37 °C (420 and 370). At 41 °C, no colonies were obtained with either *ts34* AEV or with *wt* AEV, probably reflecting a general inability of normal and transformed chick bone marrow cells to

Fig. 3 Kinetics of haemoglobin expression and effect of superinfection with *wt* AEV of *ts34* AEV-infected erythroblasts. Aliquots were stained with benzidine at different times after shift. In (a), cells from *ts34* AEV nonproducer erythroblasts (clone A6) were shifted from 35 to 41 °C without superinfection (○), after superinfection with RAV-2 (□) or after superinfection with *wt* AEV (RAV-2) at the day of shift (●). In (b), *ts34* AEV cells from a virus-producing erythroblast clone (C2) were shifted from 35 to 41 °C (○) or from 41 to 35 °C (●). As controls, cells were kept at 35 °C (■) or at 41 °C (□). The 41 °C control cells had been shifted from 35 to 41 °C 3 days before the experiment. In all cases, cells were kept at about 1.5×10^6 per ml in growth medium during the experimental period.



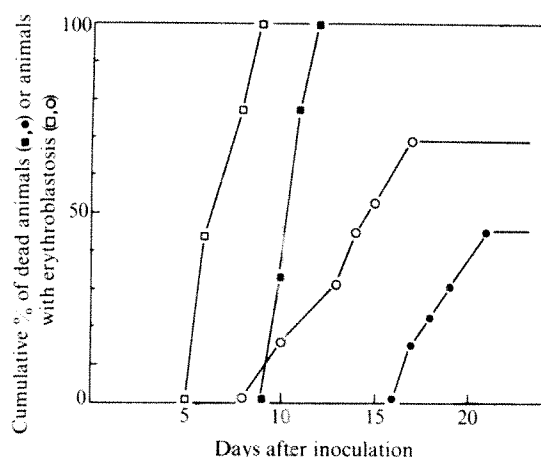


Fig. 4 Leukaemogenicity of *ts34* AEV. Three groups of 8-day old chicks were injected i.v. with 0.1 ml of growth medium containing either 1,000 colony-forming units of cell-free *ts34* AEV (RAV-2) (13 chicks); 10 colony-forming units of cell-free *wt* AEV (RAV-2) (9 chicks); or growth medium without virus (9 chicks). All animals were observed for the appearance of erythroblastosis by preparing blood smears at 2–3-day intervals and for death over a period of 3 months. Chicks with *ts34* AEV, circles; with *wt* AEV, squares. Solid symbols, cumulative % death; open symbols, % erythroblastosis.

form colonies in semisolid medium at 41 °C (unpublished observations).

The data in Fig. 4 show that *ts34* AEV is also less leukaemogenic than *wt* AEV. This was not only manifested in the decreased incidence of animals which died of leukaemia (46% as compared to 100% in control animals injected with 100 times fewer colony-forming units of *wt* AEV), but also in a delay of about 1 week in the onset of the disease and in the death of the animals. Interestingly, erythroblastosis spontaneously regressed in three of the mutant-infected chicks. We have never observed such a regression in birds infected with *wt* AEV. Two of the regressors were challenged with about 1,000 colony-forming units of *wt* AEV at 3 months of age and both died of erythroblastosis within 3 weeks post-infection. This indicates that the animals were still fully susceptible to the leukaemia and that the regression observed was probably not due to immunological defense mechanisms.

To determine whether or not the erythroblasts in the *ts34* AEV-infected animals were temperature-sensitive for haemoglobin expression, peripheral blood cells obtained from leukaemic animals were seeded at various concentrations into 15-mm tissue culture wells containing growth medium and incubated at 35 and 41 °C. Six days later foci of growing erythroblasts had developed at both temperatures and these were stained with benzidine *in situ*. The incidence of predominantly B⁺ foci was 2.5% at 35 °C (38 colonies evaluated) and 93% at 41 °C (71 colonies evaluated). In addition, B⁺ foci at 41 °C were somewhat smaller than the foci obtained at 35 °C. Similar results were also obtained with erythroblasts from two further *ts34* AEV-infected chicks, whereas only 7% of the foci at 41 °C (42 colonies evaluated) from a *wt* AEV-infected animals were B⁺.

These results indicate that *ts34* AEV is less leukaemogenic than parental AEV and that the majority of leukaemic erythroblasts from the peripheral blood of mutant-infected chicks still exhibit temperature-sensitive properties.

Discussion

The results presented here show that in erythroblasts transformed by *ts34* AEV the expression of haemoglobin can be induced by raising the temperature from 35 to 41 °C. Together with our finding that the target cell of AEV is an immature erythroid cell (ref. 5 and unpublished results) and the observation that *ts34* AEV showed a decreased leukaemogenicity *in*

vivo, this indicates that AEV induces a block of differentiation in its erythroid target cells and that this block may play a crucial part in virus-induced leukaemogenesis. Preliminary findings showed that *ts34* AEV is still capable of transforming fibroblasts at 41 °C. ARSO, AEV does not possess the *src* gene responsible for sarcoma formation by avian sarcoma viruses⁷. This raises the possibility that virus-induced leukaemogenesis is a process different from fibroblast transformation by avian sarcoma viruses. In this regard it will be interesting to determine whether transformation parameters as defined in the fibroblast system⁶ are also expressed in *ts34* AEV-transformed erythroblasts at 35 °C but not at 41 °C.

Our results are not compatible with the notion that the integration of AEV at a specific site into its host cells is sufficient to induce a leukaemic transformation. Instead, they indicate that an AEV-coded gene product has to be continuously expressed to maintain the block in differentiation and therefore also the leukaemic state of the cell. Whether this gene product is a protein and whether a host function has to be expressed in addition to it remains to be determined. A candidate for an AEV-coded protein responsible for leukaemic transformation is a 75,000 molecular weight polypeptide synthesised in AEV-nonproducer erythroblasts and fibroblasts⁹. This protein consists of part of the *gag* gene of the virus and an unknown portion with a MW of at least 55,000 (ref. 9).

In experiments in which *ts*-mutant-infected erythroblast clones were tested at rather high passages (after ~25 population doublings) only a fraction of the cells from certain clones were inducible and also had an increased tendency to lyse after shift to 41 °C (see Table 1, expt 2 and unpublished observations). Such 'aged' clones infected with *wt* AEV showed also a slight increase in haemoglobin expression after shift to 41 °C (see Table 1, expt 2 and Table 2) and we believe that this reflects a property of the host cells and not of the virus.

The *in vivo* studies with *ts34* AEV clearly show that it is less leukaemogenic than the parental *wt* AEV. Its leakiness is probably due both to its residual ability to block differentiation at 41 °C as suggested by the low levels of haemoglobin expression in some *ts34* AEV-erythroblast clones *in vitro* as well as to the selection of wild-type revertants *in vivo*. A revertant of *ts34* AEV has indeed been isolated from erythroblasts obtained from a *ts34* AEV-infected animal (unpublished results). Interestingly, the well characterised *ts68* mutant of Rous sarcoma virus appears to have an even higher residual oncogenic potential than *ts34* AEV¹⁷.

The observed 'differentiation' of *ts34* AEV-erythroblasts in response to upshift for 3 days was clearly incomplete in that the highest haemoglobin levels expressed in these cells did not exceed 8% of the amount present in mature erythrocytes. This raises the question as to whether the observed effects truly represent a step within normal erythroid differentiation. However, the conditions for induction tested may not yet have been optimal. Thus, in preliminary experiments in which *ts34* AEV erythroblasts were shifted for 8 days to 41 °C, levels of haemoglobin corresponding to about 25% of the amount present in erythrocytes were found. In addition, it might be possible to improve the physiological conditions required for optimal induction and to isolate less leaky mutants of AEV.

Obviously, the exact nature of the AEV-induced block in differentiation needs further clarification such as by studying the influence of metabolic inhibitors on the changes in haemoglobin synthesis and by testing additional parameters of erythroid differentiation. However, it seems already safe to say that chick erythroblasts infected with *ts* mutants of AEV provide a unique new system to study erythroid differentiation *in vitro*, and this can be exploited in a similar way as the induction of differentiation of mouse erythroleukaemia cell lines infected with Friend leukaemia virus by treatment with chemicals¹⁸. In addition, the AEV system has the advantage over the Friend system that possible side effects of chemicals can be avoided and that different diploid clones of 'transformed' erythroblasts can be analysed shortly after infection.

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A two-step fibrinogen–fibrin transition in blood coagulation

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The kinetics of the thrombin-catalysed release of fibrinopeptides A and B from human fibrinogen have been investigated and a mechanism correlating the release of fibrinopeptides to fibrin formation is presented. The sequential release of fibrinopeptides results in sequential activation of two sets of polymerisation sites.

WHEN thrombin catalyses the release of fibrinopeptides A and B (FPA and FPB) from mammalian fibrinogen, FPA must appear^{1–5}, followed, after a lag phase, by FPB, for polymerisation to occur. Preferential release of FPB by an enzyme in copperhead snake venom (*Agkistrodon contortrix*) does not result in normal polymer formation unless FPA is also removed^{6,7}. Light-scattering studies^{8,9} have demonstrated two types of initial polymerisation geometry of thrombin-activated fibrinogen. At near-neutral pH the geometry is predominantly end-to-end followed by lateral aggregation⁸. Fibrin formed when FPA is released has a lower mass-to-length ratio than that formed after release of both FPA and FPB¹⁰. Thus, it has been proposed that two sets of polymerisation sites are involved in fibrin formation^{10–12}: one set, A:a, is activated on release of FPA and results in end-to-end polymerisation; the other, B:b, is activated on release of FPB and results in lateral aggregation. Shen *et al.*¹⁶ speculated that the lateral aggregation following release of FPB may be of particular importance for rapid growth of the fibrin fibre. There is direct experimental evidence for at least one set of binding sites, probably the proposed A:a site^{13–15}. Here we report evidence for the existence of the B:b binding sites and that interaction at these sites is responsible for the enhancement of release of FPB observed during fibrin formation¹⁷.

Fibrin formation with and without Ca²⁺

Thrombin cleaves the bonds Aα16Arg–17Gly and Bβ14Arg–15Gly in human fibrinogen. A third bond, Aα19Arg–20Val, is cleaved more slowly¹⁸. Removal of FPA and FPB uncovers Gly as N-terminal residue on both α- and β-chains, but the γ-chain terminus is Tyr. We used N-terminal analysis to estimate the release of FPA and FPB and the cleavage of the third bond using bovine thrombin. These results were corroborated by radioimmunoassay (RIA) for FPA and FPB^{4,5}. Polymerisation was measured by ultraviolet absorption at 450 nm. Control experiments with human thrombin (3,000 NIH units per mg) showed no significant difference. Figure 1a shows the results of N-terminal analysis on thrombin digests of human fibrinogen. The

lower part of this curve corresponds to release of FPA³ which is followed by gel formation, and the upper part is due to the release of FPB³. There was no significant change in the amount of N-terminal valine throughout the digestion.

In similar experiments, FPA and FPB were determined by RIA (Fig. 2a–c). As in previous studies^{2–5}, at all pH values FPA was released first, followed by FPB after a lag phase during which polymerisation began. Rate of release, especially of FPB, was greater at higher pH and the lag phase became shorter. This effect was more evident when lower concentrations of thrombin were used. The gel point occurred when no (pH 6.3) or little (pH 7.4 and 9.0) FPB had been released. At pH 9.0 the gel was transparent; below pH 8.4–8.5 it was opaque, in agreement with the findings of Ferry and Morrisson¹⁹.

Ca²⁺ binds to fibrinogen, shortening the clotting time²⁰, and chelating agents strongly inhibit fibrin formation^{21,22}. We found that the rate of release of the two peptides at different pH values was essentially unaffected by Ca²⁺ (20 mM) and the transition from an opaque to a transparent clot was not observed in the pH range studied.

Release when polymerisation is impaired

Because the release of FPB seemed to be greatly accelerated after the formation of a clot (Figs 1 and 2), we studied its release in systems where polymerisation was impaired. Fibrinogen was digested in 2 M urea and proteolysis was assessed by N-terminal analyses (Fig. 1b). The ratio of Gly:Tyr of 1:1 suggested that only one fibrinopeptide was released when botroxobin fibrin, which is devoid of FPA³, was digested with thrombin (Fig. 1c), and the slow appearance of N-terminal Gly indicated the slow release of FPB. When a non-polymerising N-terminal fragment of fibrinogen (N-DSK)²³ was used as substrate in the absence of urea (Fig. 1d), again only one fibrinopeptide seemed to be released. When N-DSK from botroxobin fibrin was digested, in the absence of urea, the result was similar to that in Fig. 1c.

Digestion of fibrinogen (60 μM) with thrombin in 2 M urea was repeated and the release of FPA and FPB was followed by RIA (Fig. 3a). FPA was released completely but only small amounts of FPB were released and no absorbance increase at 450 nm was observed, indicating that polymerisation had not occurred. When the digest was diluted six times with buffer (pH 7.4) a polymer was formed almost instantaneously and FPB was released rapidly (Fig. 3b). To show that this was not a consequence of changes in fibrinogen or thrombin, caused by urea or on dilution of the urea solution, fibrinogen (60 μM) in 2 M urea was kept for 1 h at 37°C and then diluted with buffer containing thrombin. On dilution, polymerisation occurred and

the sequential release of FPA and FPB was the same as with fibrinogen not pretreated with urea. In a second control experiment, thrombin (0.95 NIH units ml^{-1}) was incubated in 2 M urea for 1 h and then diluted in buffered fibrinogen to give a solution which was 0.33 M in urea and 7 μM in fibrinogen (pH 7.4), and containing 0.16 NIH units of thrombin per ml. Polymerisation occurred in this system and the pattern of release of FPA and FPB was the same as for native fibrinogen at similar thrombin concentrations. Other control experiments were carried out with derivatives of fibrinogen having no capacity to polymerise; botroxobin-treated N-DSK (devoid of FPA but containing FPB) was digested with thrombin in 2 M urea (Fig. 3c). No substantial amounts of FPB were released, but on dilution, it was released slowly (Fig. 3d). FPB was released from polymerising fibrin (Fig. 3b) much faster than from N-DSK in

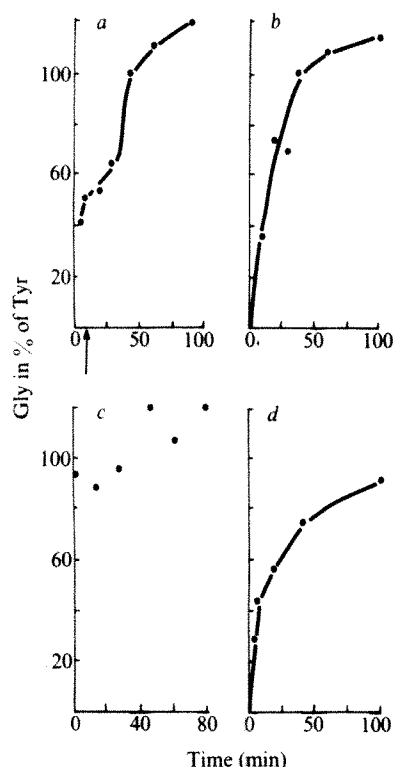


Fig. 1 Appearance of N-terminal glycine during digestion with thrombin of fibrinogen and derivatives of fibrinogen. Human fibrinogen (95% clottable), fibrinogen and fibrin N-DSK preparations were as described previously^{23,35}. Bovine thrombin, 1,800 NIH units per mg, was purified as described elsewhere³⁵. Botroxobin fibrin was prepared by adding 10 μl of an aqueous solution of botroxobin (from *Botroxobin B marajonesis*, 2,400 BU per mg, Pentapharm, 0.5 mg ml^{-1}) to 6 ml of 1% solution of fibrinogen in 0.15 M NaCl. The solution was incubated at 37 °C for 4 h. The fibrin clot was dissolved in 6 ml 4 M urea and dialysed at +4 °C against 25 mM Tris-HCl, pH 7.2, 2 M in urea and 0.125 M in NaCl. The fibrin solution was diluted to 20 μM with the same buffer and stored at -20 °C in 2 ml portions. Substrates (20 μM) were digested with thrombin at 37 °C in 25 mM Tris-HCl, 0.125 M NaCl, pH 7.2 (where indicated, the medium was 2 M in urea). The procedures used in following the hydrolysis have been described in detail³⁵, 'method A' being used for substrates b and c and 'method B' for substrate d; for substrate a the procedures were modified because fibrin clot formation prevented removal of a representative aliquot of the digest. This problem was circumvented by carrying out the digestion in eight tubes, one tube for each assay time, and thereafter carrying out N-terminal analysis on the contents of each tube according to method A³⁵. a, Fibrinogen, thrombin = 0.18 NIH units ml^{-1} . Arrow indicates the time of formation of a solid gel; b, fibrinogen in 2 M urea, thrombin = 0.82 NIH units ml^{-1} ; c, botroxobin fibrin in 2 M urea, thrombin = 4.0 NIH units ml^{-1} ; d, fibrinogen N-DSK, thrombin = 0.18 NIH units ml^{-1} .

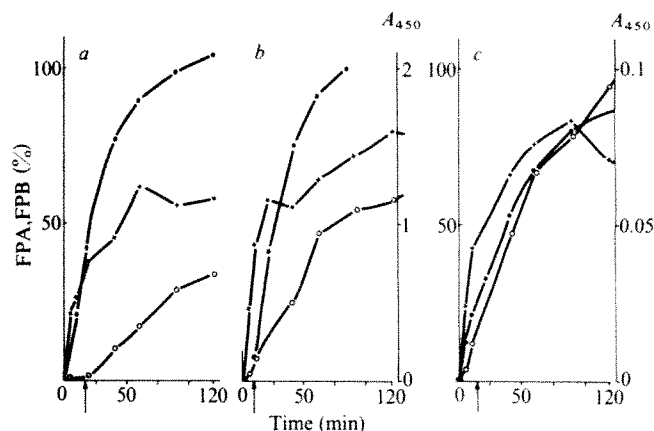


Fig. 2 Thrombin-induced release of FPA and FPB from fibrinogen as determined by radioimmunoassay (RIA) technique. Human fibrinogen (93–100% coagulability) was diluted in Tris-imidazole-HCl buffer⁸ (pH 6.35, 7.4 or 9.0) to give a final solution 17 mM in Tris and imidazole, ionic strength 0.15 and protein concentration 8–10 μM . Fibrinopeptide assay: the fibrinogen solutions were prewarmed at 37 °C. Aliquots of 200 μl were placed in eight siliconised tubes. Bovine thrombin (specific activity 200 NIH units per mg) was added to a final concentration of 0.025 or 0.05 NIH units ml^{-1} and incubated at 37 °C. The digestion was interrupted by adding 0.1–0.2 AT units of hirudin (Pentapharm) at different incubation times. Urea (200 μl of 8 M) was added to dissolve clots formed or, in some cases, the clots were removed before analysis. Radioimmunoassay (RIA) of FPA and FPB was carried out as described elsewhere^{4,5}. Polymerisation: As each of the above samples was taken for RIA determination, a separate prewarmed sample of 1 ml was put into a quartz cuvette and thrombin was immediately added to the same concentration as above. Optical density at 450 nm against time was monitored in an Acta C III spectrophotometer (LKB-Beckman). The transparent type of clot (see Fig. 2c) was not formed in the presence of Ca^{2+} . The time required for formation of a solid gel is indicated by arrows. The latter determinations were carried out in separate tubes. a, pH 6.35; b, pH 7.4; c, pH 9.0. ●, A_{450} ; ×, FPA; ○, FPB.

the same conditions. We believe that the polymer formed on dilution of thrombin-treated fibrinogen in urea solution represents a physiological fibrin structure, because it subsequently cross-linked in the presence of factor XIII_a to give a polyacrylamide gel pattern (Fig. 3, insert) indistinguishable from that produced when fibrinogen, in the absence of urea, was clotted with thrombin in the presence of factor XIII_a and Ca^{2+} .

Another derivative of fibrinogen with impaired gel-forming capacity is obtained by photooxidation²⁴. When photooxidised fibrinogen was exposed to thrombin, FPA only was released rapidly. When this substrate was digested with thrombin in 2 M urea, FPA was released fast and FPB slowly. On dilution of this system, the release of FPB was only slightly increased.

We next investigated congenitally dysfunctional fibrinogen from individuals homozygous and heterozygous for dysfibrinogenemia Detroit, in which fibrin formation is delayed^{25–27}. In this condition FPA is released at an almost normal rate, but the release of FPB is slow. When normal and affected fibrinogen were exposed to thrombin (Fig. 4a–c) the release of FPA was always fast. In homozygous fibrinogen Detroit no polymer formation was observed during FPA release and the initial release of FPB was slow (Fig. 4c). After prolonged incubation, polymerisation occurred and the release of FPB was accelerated concomitantly (Fig. 4c). Heterozygous fibrinogen Detroit (Fig. 4b) gave the same patterns as normal fibrinogen (Fig. 4a), as expected, because the heterozygous fibrinogen contains about 50% 'normal' fibrinogen^{25,27}, providing an initial concentration of 'normal' A α -chain which is almost twice the K_m for thrombin-catalysed cleavage of this domain in fibrinogen²⁸.

To demonstrate the causal link between delayed polymerisation and the release of FPB, fibrinogen Detroit was

digested with botroxobin (Fig. 5b). FPA was released but polymerisation and release of FPB occurred only when thrombin was added (Fig. 5c).

Sequence of events in formation of fibrin

It is thought that one binding domain (A) becomes active after release of FPA and is located in the N-DSK region of fibrinogen¹³⁻¹⁵. This domain interacts with a C-terminal domain (a) located in a plasmin fragment of fibrinogen, fragment D. In

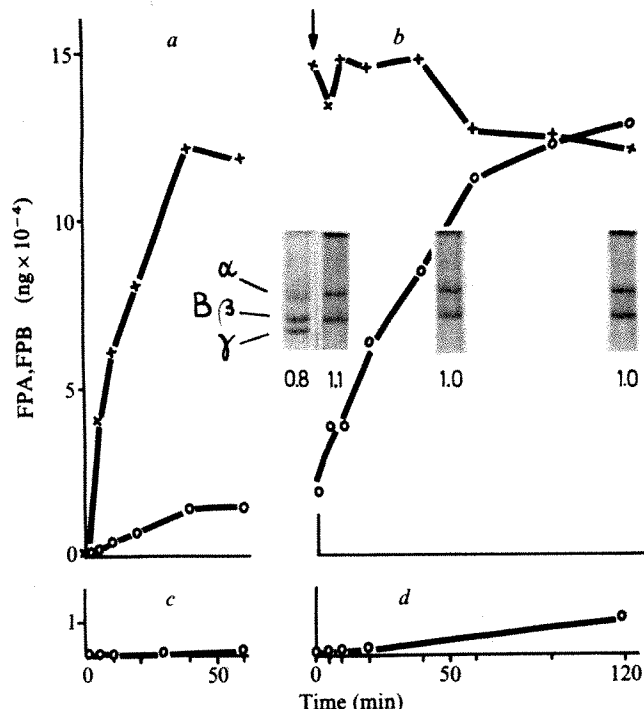


Fig. 3 Thrombin-induced release of FPA and FPB from fibrinogen and N-DSK in urea. *a*, Fibrinogen (see legend to Fig. 2) was reconstituted in 2 M urea to give a solution 0.3 M in NaCl and 60 μ M in protein. This solution (pH $\sim$ 7) was incubated at 37°C with bovine thrombin (200 units per mg) at a concentration of 0.8 NIH units per ml. After various time intervals, aliquots (200 μ l) were taken and thrombin quenched with hirudin (1.0 AT units). RIA was carried out as described in the legend to Fig. 2. *b*, After digestion for 1 h the bulk of the digest was used as follows: 50- μ l aliquots were transferred to a series of siliconised glass tubes. A sixfold dilution was obtained by adding 250 μ l of 17 mM Tris-imidazole-HCl buffer, pH 7.4, containing 24 mM calcium acetate, 2.5 mg factor XIII (Behringwerke) and 1 μ l of Trasylol (Bayer), containing 1,500 KIE per ml, and digests were incubated at 37°C for various times. The reaction was interrupted by adding hirudin (0.5 AT units). Clots (arrow) were formed after dilution and these were removed from one set of experiments for SDS-gel electrophoresis (see inserts). The gel furthest to the left shows the digest, before dilution, contains non-cross-linked α , B β and γ -chains. In the other gels poly α (at the top) and γ - γ dimers (just above α chain) are seen. The clots from another set were dissolved (8 M urea in 0.2 M NaOH) and protein determined at 282 nm using $\epsilon = 5.1 \times 10^{-5}$ (ref. 24). The amount (mg) of protein obtained is shown at the bottom of the SDS gels. FPA and FPB were determined on the clot supernatants using the RIA technique as described in Fig. 2. *c*, N-terminal fragment of fibrinogen (N-DSK)²⁹ was used as substrate. 20 mg of N-DSK in 2 ml of 0.2 M NH_4HCO_3 , pH 7.9, was digested (3 h, 37°C) with 0.2 BU of botroxobin. Botroxobin N-DSK, devoid of FPA, was isolated by gel filtration (Sephadex G-25) in 0.1 M NH_4HCO_3 . Botroxobin N-DSK (60 μ M) in 2 M urea containing 20 mM calcium acetate, was incubated for 60 min at 37°C with thrombin (0.8 NIH units ml^{-1}). FPA and FPB were determined as described in *a*. *d*, Dilution of the botroxobin-N-DSK-solutions was the same as described in *b*. $\times$, FPA; $\circ$, FPB.

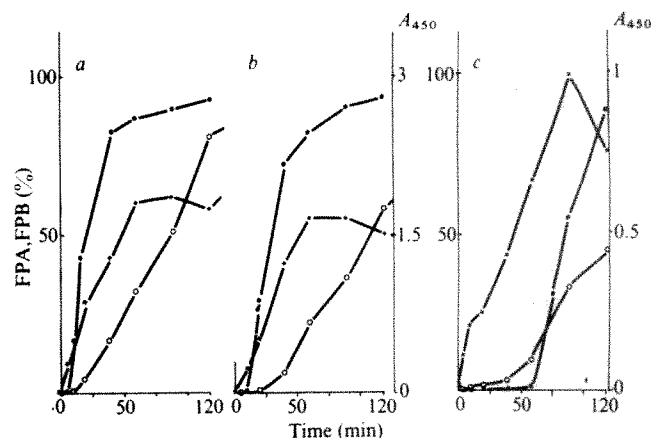


Fig. 4 Release of fibrinopeptides and polymerisation in normal and abnormal fibrinogens. Fibrinogens (fraction I-2F) from normal plasma and from two members (heterozygote and homozygote) of a family afflicted with dysfibrinogenemia Detroit were prepared as previously described, omitting the ammonium sulphate precipitation step²⁵. Stock solutions of fibrinogen (60 μ M) in 0.3 M NaCl were prepared. They were diluted with buffers at pH 7.4, containing Tris-imidazole-HCl and calcium acetate to give solutions which were 10 μ M in fibrinogen, 17 mM in Tris-imidazole and 20 mM in calcium acetate. To aliquots (200 μ l) of these solutions 1 μ l of Trasylol (1KIE) was added. Bovine thrombin was added to a concentration of 0.025 NIH units per ml. Incubation was carried out at 37°C for various times and the digestion was interrupted by addition of hirudin (0.1 AT units). Polymerisation was determined as described in the legend to Fig. 2 except that no 'gel time' determination was made. Fibrinopeptides were determined by RIA as described in the legend to Fig. 2. *a*, Normal fibrinogen; *b*, fibrinogen Detroit, heterozygote (S.H.)^{26,27}. *c*, fibrinogen Detroit, homozygote (P.H.)^{26,27}. $\times$, FPA; $\circ$, FPB; $\bullet$, A_{450} .

fibrin formation, an activated A domain of one molecule probably interacts with an A domain of another. Evidence for another set of binding sites comes from our demonstration that the release of FPA from fibrinogen Detroit fails to produce polymerisation, although polymerisation does occur eventually after release of FPB. The A α sites of fibrinogen Detroit are apparently not activated due to the mutation²⁵ A α 19Arg $\rightarrow$ Ser. This mutation, three amino acid residues away from the thrombin-susceptible residue (A α 16Arg) in the A α -chain, would be expected to produce conformational changes in this part of the molecule affecting subsequent interactions²⁹. We propose that the release of FPB activates a polymerisation domain (B) which interacts with another site (b) in a neighbouring molecule. Binding studies¹⁵ with thrombin-activated fibrinogen Detroit coupled to Sepharose have shown that conjugates lacking FPA as well as FPB have no affinity for normal fibrinogen. This puzzling observation could be explained if one polymerisation domain is left intact in fibrinogen Detroit, and is inactivated by the coupling of fibrinogen to Sepharose.

Our results support the suggestion²⁻⁵ that prior release of FPA is essential for the release of FPB in the presence of thrombin. Selective enzymatic removal of FPB from fibrinogen does not produce polymerisation at 37°C unless FPA is also released^{7,8}, demonstrating that the unfolding of the B:b polymerisation sites is linked with the release of FPA. It is interesting that polymer formation greatly accelerates the release of FPB. The experiments with fibrinogen Detroit strongly suggest that this effect is brought about by interactions at the B:b sites. Such interaction could induce a conformational change in a nearby B β -chain favourable to the hydrolysis of the B β 14Arg-15Gly bond by thrombin. The overall consequence of increased FPB release is an acceleration of polymer formation, an effect relevant to any discussion of thrombus formation *in vivo*.

Our results have a bearing on the role of calcium in fibrin formation. It seems that Ca^{2+} , which has only a minor effect on

the activation process itself, shortens the time for subsequent gel formation. This may account for the effect of Ca^{2+} on the opacity of clots. Complete transition from opaque to transparent clots occurs in the absence of Ca^{2+} at pH 8.5. In the presence of Ca^{2+} opaque clots are produced throughout the pH range that has been studied, suggesting that Ca^{2+} influences the ionisation of residues with $pK_s \leq 8.5$. Ca^{2+} ions possibly stabilise a conformation favourable to the formation of opaque clots, and they are known to bind to fibrinogen with high affinity at three binding sites³⁰ and to protect against thermal denaturation³¹.

Light-scattering data⁸ can be interpreted in terms of interacting domains. Those data show that the initial polymerisation geometry at pH 6–7 is predominantly 'end-to-end'⁸. At the lower end of this range, polymerisation is almost exclusively correlated to FPA release and the end-to-end geometry probably results from activation of the A:a binding sites. At pH 9–10, when lateral aggregation is a dominant feature of initial polymerisation geometry⁸, release of FPB is particularly fast and the lag phase is short. Lateral aggregation can therefore be explained by activation of the B:b binding sites after release of FPB. However, the release of FPA is also fast at high pH, indicating that the interaction at the A:a sites is hampered at high pH.

The possible mechanism of fibrin formation *in vivo*

Figure 6 represents the sequence of events in fibrin formation. If the B sites are close to FPB in the dimer of fibrinogen, an interaction involving the B site on one side of the dimer with its corresponding b site can modify the structure of the 'free' B site on the other half-molecule, so that for steric or conformational reasons the B peptide is cleaved faster by thrombin.

Our results are relevant to fibrin formation *in vivo*. The slow initial release of FPB and the presence of antithrombins in blood implies that type I fibrin is the favoured species in blood. Some indications of this were obtained in preliminary experiments with fibrin isolated from clotted whole blood. Blood was drawn into glass bottles containing inhibitors of fibrinolysis (Trasylol, 40 KIE ml^{-1} and ϵ -aminocaproic acid, 2 mg ml^{-1}). The blood

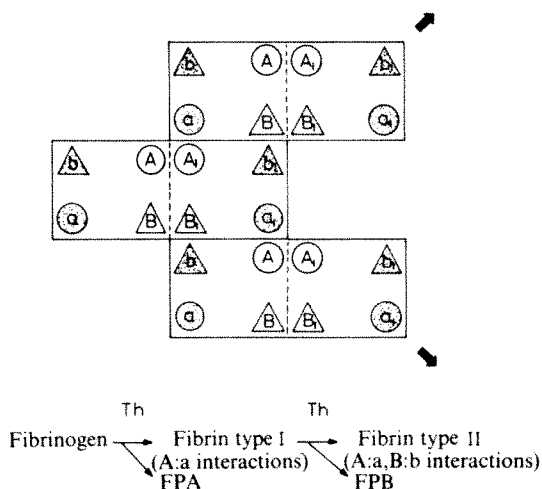


Fig. 6 Schematic representation of sequence of events in fibrin formation. The upper half of the figure shows schematically possible interactions at the sites A:a and B:b. Because of the dimeric nature of fibrinogen each of these sites is present in duplicate, that is, AA₁ and so on. The arrows show that the direction of the initial growth of the polymer is different when the A:a or the B:b sites are exclusively activated. The lower part of the picture summarises the events which occur during formation of the two types of fibrin.

was allowed to clot for 6 h at 37°C and then left overnight at +4°C. The fibrin was isolated by washing extensively with water and buffered glycine solution (pH 6.9) followed by extractions with chloroform-methanol (2:1) and ethanol (30%). The fibrin, highly cross-linked as judged by SDS-gel electrophoresis, was cleaved with CNBr and the N-DSK portion was isolated²³. SDS-gel electrophoresis showed that the purity of the N-DSK isolated from blood fibrin was the same as that of N-DSK prepared from purified fibrinogen. N-terminal analysis of N-DSK from blood fibrin showed a Gly/Tyr ratio of about 1.3:1 compared with the expected 2:1 if both FPA and FPB had been released. When N-DSK from blood fibrin was digested with thrombin (80 NIH units ml^{-1}) the change in N-terminal pattern suggested cleavage of the B β 14Arg-15Gly bond, indicating that FPB was part of the N-DSK structure in this material.

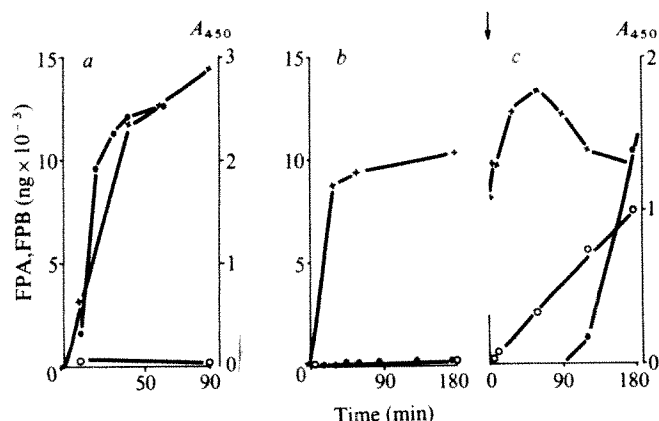
Type II fibrin may therefore form only at sites within blood vessels where the thrombin concentration is extremely high, that is, at the site of a lesion in the vessel wall where adhesion and aggregation of platelets occurs. Near the lesion, thrombin concentration rapidly decreases due to blood flow and interaction with antithrombin, and the fibrin formed is probably mainly type I. However, even a slight activation of the B site with subsequent B:b interaction may considerably change the subsequent time course of fibrin formation due to accelerated FPB release. This may result from disturbances in the composition of blood or its flow properties and may predispose it to the development of occluding thrombi in arteries and veins. It is interesting that type II fibrin seems to be more compact than type I¹⁰ and that the latter is more easily digested by plasmin³². Type I fibrin may then more easily form soluble complexes with fibrinogen which are subsequently attacked rapidly by plasmin^{33,34}.

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Fig. 5 Release of fibrinopeptides and polymerisation in normal and abnormal fibrinogen. Fibrinogens were prepared and diluted as described in the legend to Fig. 4. RIA and polymerisation curves were determined as described in the legend to Fig. 2. *a*, Normal fibrinogen. Digestion with botroxobin was carried out at a concentration of 0.02 BU per ml at 37°C. A firm clot was formed which could not be dispersed in the buffer used. Therefore, subsequent addition of thrombin gave only partial release of FPB (not shown). *b*, Fibrinogen Detroit, homozygote. Digestion with botroxobin carried out as in (*a*). *c*, Fibrinogen Detroit, homozygote, in a separate experiment was digested with botroxobin in the same conditions as in (*a*) for 100 min. At that time (arrow), thrombin was added to a concentration of 0.025 NIH units per ml and incubated at 37°C for an additional 3 h. $\times$, FPA; $\circ$, FPB; $\bullet$, A_{450} .



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Synthesis of an ovalbumin-like protein by *Escherichia coli* K12 harbouring a recombinant plasmid

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A cloned DNA transcript of ovalbumin mRNA was cut a few nucleotides away from the initiator codon, and fused in phase to the beginning of the Escherichia coli β -galactosidase gene. The hybrid gene has been cloned in E. coli where it produces large amounts of an ovalbumin-like protein.

RECOMBINANT DNA technology now permits the introduction and cloning, in *Escherichia coli* K12, of genes of various origins. However, the expression of foreign genes in this bacterial environment is still poorly documented. Several genes isolated from the lower eukaryotes (yeast, *Neurospora crassa*, *Drosophila*) have been shown to be spontaneously expressed in *E. coli*^{1–6}. There has been no report of genes cloned from the higher eukaryotes that are spontaneously transcribed or translated. Transcription can be efficiently enforced by the integration of gene sequences within a bacterial or a phage gene^{7,8}. It seems that the best way to promote translation into a polypeptide product is by the construction of hybrid genes in which the eukaryotic sequence is fused in phase to a bacterial gene. This approach has been used to construct *E. coli* strains capable of producing the small hormone, somatostatin, in the form of a hybrid protein⁹. The gene coding for the hormone, which is 14 amino acids long, was synthesised chemically, fused to the end of the *E. coli lac Z* gene coding for β -galactosidase and cloned in a multicopy plasmid. The β -galactosidase moiety (about 1,000 amino acids) was elegantly connected to the somatostatin moiety through a methionine residue. As somatostatin contains no methionine, and as the latter is labile in certain conditions, the hormone could be extracted from the hybrid polypeptide by treatment with cyanogen bromide⁹.

Most polypeptides, however, contain methionine residues, so that the above method is not of potential general use unless

other cleavable protein 'linkers' are made available. Many possible applications of genetic engineering, on the other hand, rely on the synthesis of polypeptides larger than somatostatin, coded by gene sequences too long to be chemically synthesised at present. The recent discovery that at least some eukaryotic genes are split^{10–20} makes it unlikely that such genes, isolated from the genome, are easily amenable to expression in *E. coli*. Rather, the most general approach seems to involve (1) enzymatic synthesis of the desired gene sequence from mRNA; (2) fusion of this cDNA sequence to a bacterial gene in such a way that the eukaryotic sequence can be read in the proper phase; (3) cloning in *E. coli*.

We have applied this protocol to the chicken ovalbumin gene sequence and we report here the construction of bacterial strains which synthesise large amounts of an ovalbumin-like protein. This result demonstrates the practicability of the approach and opens broader prospects to genetic engineering methodology.

The ovalbumin system

Ovalbumin is the major egg white protein. It is a polypeptide chain of 386 amino acids, with a single intra-chain disulphide bond, and undergoes a few post-translational modifications such as cleavage of the first methionine residue, N-acetylation, glycosylation and phosphorylation of certain residues. There are several known genetic variants^{21–26}.

Ovalbumin mRNA (ov mRNA) is predominant in the chick oviduct, which facilitates its isolation in relatively pure form. Ov mRNA is 1,859 nucleotides long. Its complete sequence has been determined by McReynolds *et al.*^{26,27}, and consists of a 64-nucleotide long 5' non-coding region, followed by a 1,158-long coding region, and a 637-long 3' non-coding region (Fig. 1).

The ovalbumin gene is split in the chicken genome^{12,16,20}. We have recently isolated, by molecular cloning, the various genomic DNA fragments which code for cytoplasmic ovalbumin mRNA^{16,17}. Analysis of the cloned fragments has revealed the presence of at least six intervening sequences, all located within translated regions of ov mRNA. As the structure of the

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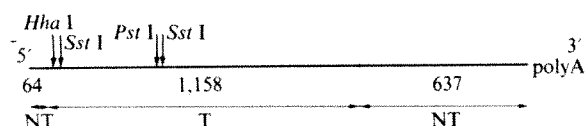


Fig. 1 Map of ovalbumin mRNA. Schematic representation of ov mRNA according to McReynolds *et al.*²⁶, showing the translated (T) and non-translated (NT) regions, the AUG start signal (nucleotides 65-67) for protein synthesis, and the position of the *HhaI*, *PstI* and *SstI* sites in the corresponding DNA sequence^{26,31}.

ovalbumin gene was likely to prevent its expression in *E. coli*, we turned our attention towards the DNA transcript of ov mRNA.

Humphries *et al.*²⁸, using a protocol devised for the cloning of globin mRNA transcripts^{29,30}, first isolated a plasmid, pCRI ov2-1, which carries about 1,730 base pairs of DNA transcribed from ov mRNA. Evidence has been presented that a faithful cloned DNA copy is obtained²⁸. As ov mRNA is 1,859 nucleotides long, pCRI ov2-1 does not harbour all the ov mRNA sequence. Restriction mapping, DNA sequencing and electron microscopy analysis^{16,17,28,31}, however, indicate that the missing parts originate from untranslated regions of the mRNA.

As pCRIov2-1 carries all the sequence coding for ovalbumin, we first asked whether the gene is expressed in C600r_m⁺ (pCRIov2-1). Transcription of the ovalbumin sequence from the correct strand could be demonstrated. However, the strain produced no ovalbumin-like protein, as indicated by radioimmunoassays (see below) which were negative, even in conditions where less than one molecule per cell would have been detected (O.M.-P., unpublished). We tentatively concluded that the ovalbumin sequence in pCRIov2-1 has been accidentally fused to an unknown plasmid gene, probably out of the proper reading frame.

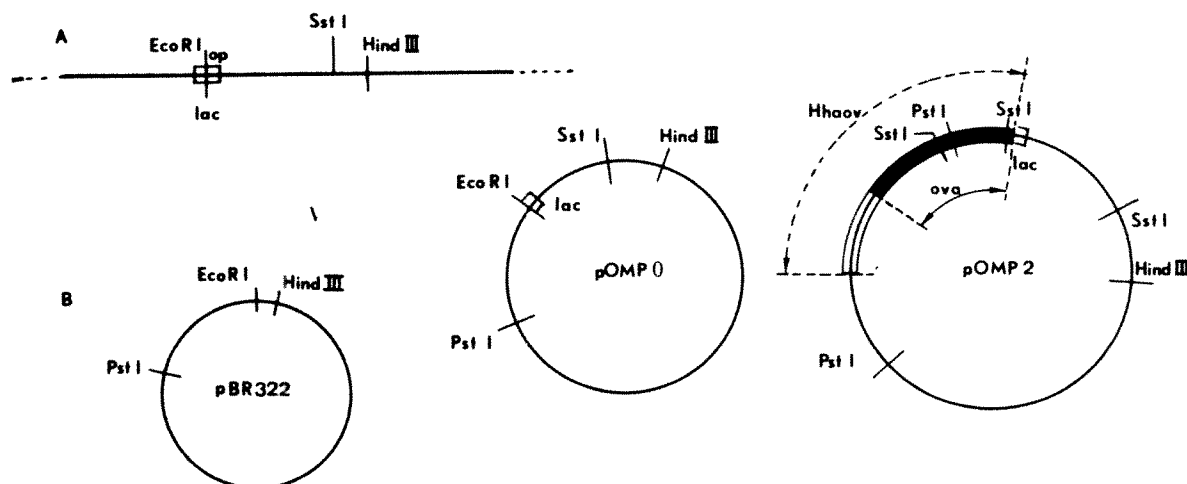
Construction of recombinant *lac*-ov plasmids

We then decided to fuse the ovalbumin gene sequence to the beginning of the *E. coli lac Z* gene, which codes for β -galactosidase. Fuller³² has generated a small DNA fragment of 205 base pairs, flanked by two *EcoRI* sites, which carries, in addition

to the L8UV5 *lac* operator promoter region, the information for the first eight amino acids of β -galactosidase. (The L8UV5 mutation makes the system insensitive to catabolite repression.) This fragment was engineered by Charnay *et al.*³³ into a phage, λ cl857plac5 Δ ZL8UV5, the DNA of which has a single *EcoRI* site located after the eighth amino acid of β -galactosidase in the *lac* sequence. We used this DNA to generate by digestion with *EcoRI* and *HindIII* a DNA fragment of 2.85 kilobases which carries the beginning of the *lac* sequence. This fragment was recombined *in vitro* with pBR322 DNA³⁴ digested by the same two enzymes, as outlined in Fig. 2, and cloned in *E. coli*. The resulting plasmid, called pOMP0, was the vector used for further recombination experiments. pOMP0 is about 7.1 kilobases in size. It makes the recipient host cell resistant to ampicillin (but not to tetracycline) and *lac* constitutive (because *lac* repressor is overtitrated by the operator carried by the multicopy plasmid), and has a single *EcoRI* site located near the beginning of the *lac Z* gene (Fig. 2).

Most of the ovalbumin sequence carried by plasmid pCRIov2-1 can be isolated in a restriction fragment of 2,430 base pairs, *Hhaov*, obtained by digestion of plasmid DNA with *HhaI*^{12,28}. The analysis of cloned genomic parts of the ovalbumin gene¹⁷ has demonstrated the existence, near the 5' end, of a site sensitive to *HhaI*, located very close (about 30 base pairs) to an *SstI* site in a region present in pCRIov2-1 (ref. 31). When the nucleotide sequence of this region of ov mRNA established by McReynolds *et al.*²⁶ became available, it seemed that there was no *HhaI* site (GCGC) in the sequence. It occurred to us, however, that 35 nucleotides ahead of the *SstI* site closest to the 5' end (nucleotides 116-120), there was a sequence, GGUGCA (nucleotides 77-82), which could be changed to GGCGCA without modifying the amino acid sequence (GGU and GGC both code for glycine). The assumption could be made that McReynolds *et al.*²⁶ and Humphries *et al.*²⁸ had worked on different genetic variants and that the *HhaI* site which we observed would be located at nucleotides 78-81 of ov mRNA, overlapping two triplets, GGC and GCA, which code for the fifth and sixth amino acids of ovalbumin. Accordingly, the fragment extracted from pCRIov2-1 should be cut at the very beginning of the mRNA sequence coding for amino acids.

Fig. 2 Construction of plasmids pOMP0 and pOMP2. λ cl857plac5L8UV5 Δ Z was constructed by Charnay *et al.*³³ by first inserting the 205-base pair *lac* sequence (described in ref. 32) into the *EcoRI* site located at the end of the *lac Z* gene of a derivative of λ cl857plac5L8UV5. Intramolecular recombination subsequently yielded a phage with only one *EcoRI* site between the end of the *lac Z* gene and the 205-base pair *lac* sequence as shown in a. 500 ng of λ cl857plac5L8UV5 Δ Z DNA (shown as a, where only the region carrying the *lac* sequence is depicted) and 50 ng of pBR322 DNA (shown as b) were digested by *EcoRI* and *HindIII*, and ligated by T4 DNA ligase in a final volume of 15 μ l as usual^{16,35}. Aliquots were used to transform CaCl₂-treated strain 478 (C600 r_m⁺recBC⁻lacy⁻) and transformants were selected on tryptone plates containing ampicillin (20 μ g ml⁻¹) and 5-bromo-4-chloro-indolylgalactoside (X-gal, 40 μ g ml⁻¹), on which strains carrying the recombinant plasmid give blue colonies. Purified pOMP0 was next used to construct recombinant plasmids harbouring the ovalbumin sequence (see legend to Fig. 3). The map of one representative plasmid, pOMP2, was established by restriction analysis using the indicated sites (see Fig. 4). pOMP0 is about 7.1 kilobases long, has single sites for *EcoRI*, *HindIII*, *SstI* and *PstI*. All experiments were carried out with ultrapure hyperhydor water (Institut Pasteur).



I	Met	Thr ¹	Met ²	Ile ³	Thr ⁴	Asp ⁵	Ser ⁶	Leu ⁷	G β-galactosidase G			
	A T G	A C C	A T G	A T T	A C G	G A T	T C A	C T G				
	T A C	T G G	T A C	T A A	T G C	C T A	A G T	G A C				
II	Ovalbumin			Met	Gly ¹	Ser ²	Ile ³	Gly ⁴	T G			
				A T G	G G C	T C C	A T C	G G C				
				T A C	C C G	A G G	T A G	C C				
III	Met	Thr	Met	Ile	Thr	Asp	Ser	Leu	Ala	Ala	Ser	Met
	A T G	A C C	A T G	A T T	A C G	G A T	T C A	C T G	C C A	G C A	A G C	A T G
	T A C	T G G	T A C	T A A	T G C	C T A	A G T	G A C	G C T	C G T	T C G	T A C

Further radioimmunoassays were carried out to quantitate the amount of ovalbumin-like product made in *E. coli*. In Fig. 5, competition by pure, unlabelled ovalbumin (Fig. 5b) is compared to competition by extracts of an *E. coli* strain carrying pOMP2 (Fig. 5a). The competition by the latter is only 80% complete, which suggests that one (or several) antigenic site is missing in the protein manufactured by bacteria. Furthermore, the competition curve may be regarded as being multiphasic. This suggests either that some of the antigenic sites made in *E. coli* are more abundant than others, or that some have a lower affinity than those of native ovalbumin. Because the size of the

ovalbumin-like product is approximately that expected (Fig. 6 and below), we favour the second interpretation. As we noted above, ovalbumin, in the oviduct, undergoes some post-translational modifications which may play an important part in the structure of antigenic determinants. As it is unlikely that such modifications take place in *E. coli*, the ovalbumin-like product (which in any case differs from native ovalbumin by a few amino acids in the N-terminal end) may be, for some of the antigenic sites, a less efficient competitor than ovalbumin. On this assumption, quantitation based on the first part of the multiphasic curve indicates that 2.5×10^6 cells may produce the equivalent of about 15 ng of ovalbumin, that is, about 90,000 molecules of ovalbumin-like protein per cell. If the whole curve was considered as monophasic the estimate would be of 45,000 molecules per cell (5,000–45,000 in a variety of independent experiments, 30,000 on average).

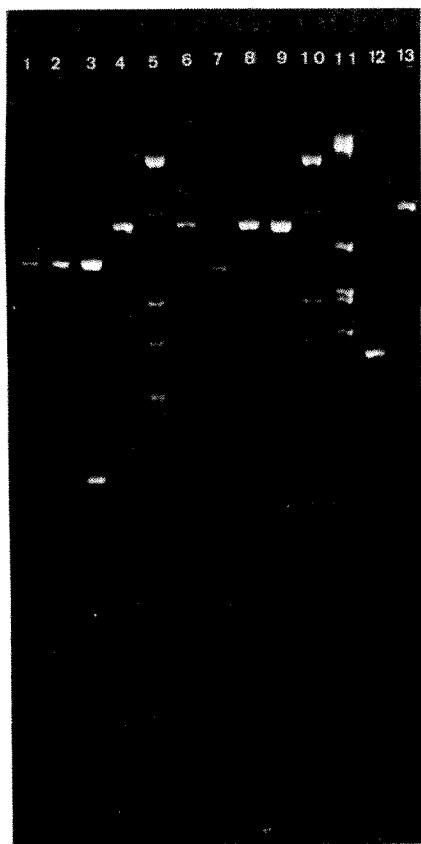


Fig. 4 Restriction analysis of plasmids carrying the ovalbumin sequence. Horizontal agarose (0.5% Sigma type II) gels (25 cm long) were run at 5 V cm^{-1} as in ref. 40. Plasmid DNAs were purified from cleared lysates by phenol extraction and ethanol precipitation, subjected to digestion by *Pst*I endonuclease (Boehringer)^{41,42}, and run in parallel with size markers (lanes 5 and 10: λ cl857Sam7 DNA digested by *Eco*RI and *Bam*HI^{43,44}, lane 11: λ cl857S7 DNA digested by *Eco*RI (ref. 43), lane 12: pBR322 DNA digested by *Hind*III (ref. 34). The size of fragments was estimated on a semilogarithmic plot relating distance to size (in kilobase pairs). Plasmids pOMP1, 2 and 4 (lanes 1, 2 and 3, respectively) show two fragments of estimated size 6.9 and 2.8 kilobases. Plasmids pOMP5, 7, 10 and 11 (lanes 4, 6, 8 and 9, respectively) show two fragments of about 8.65 and 0.7 kilobases. Plasmid pOMP9 (lane 7) does not yield the expected fragments. The total plasmid size (except for pOMP9) can thus be estimated to range over 9.35–9.8 kilobases. The theoretical size is 9.63 kilobases (using estimates of 4.35 kilobases for pBR322; 2.43 kilobases for *Hha*ov; 2.85 kilobases for the λ cl857 *Eco*RI–*Hind*III fragment (as determined by electron microscopy). The respective orientation of the *lac* and ovalbumin sequences which can be deduced from these data was confirmed, in the case of pOMP2, by a variety of other experiments including double digestion by *Pst*I and *Hind*III, which yields three fragments of about 3.45, 3.1 and 2.97 kilobases, and digestion by *Sst*I (data not shown) (see Fig. 2). Lane 13, plasmid pOMP2 digested by *Hind*III (ref. 34).

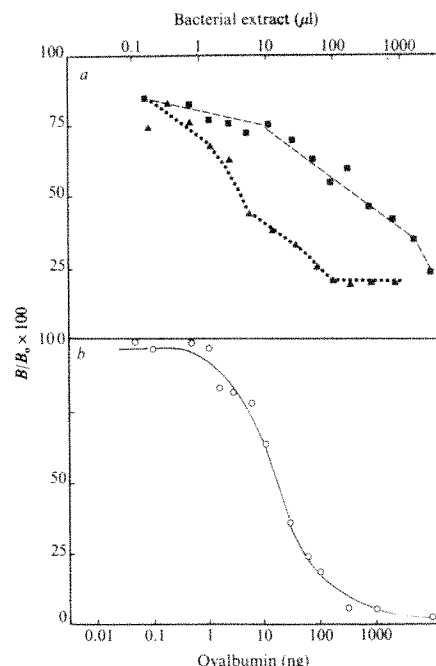
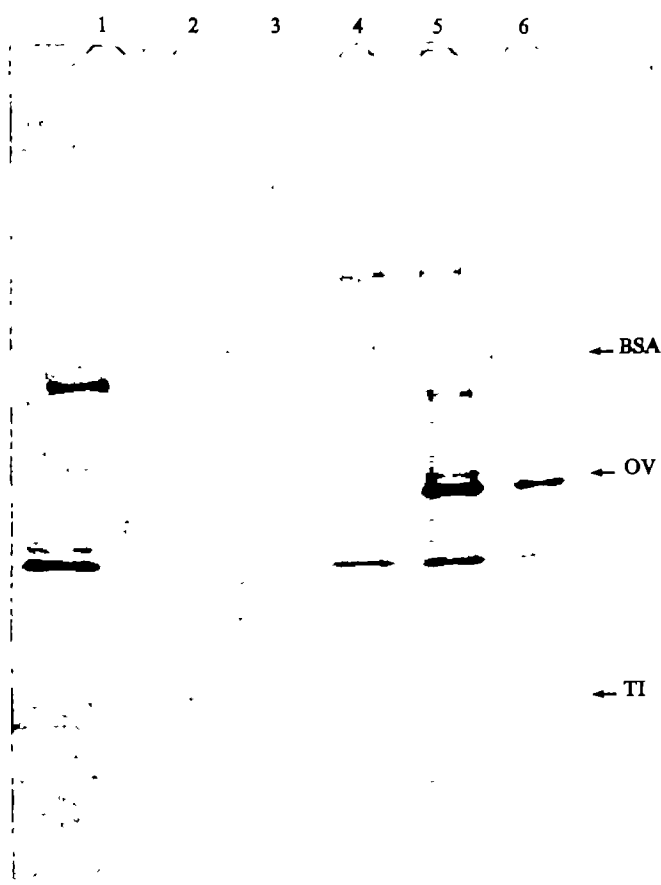


Fig. 5 Detection of ovalbumin-like protein in bacterial extracts. Ovalbumin (Sigma, 99% pure) ($30 \mu\text{g}$ in $20 \mu\text{l}$) was labelled with 1 mCi of Bolton and Hunter reagent (Amersham; $>1,375 \text{ Ci mmol}^{-1}$), according to the procedure of ref. 45, except that the reaction was stopped after 18 h at 0°C . Radioactive protein was then applied to a Sephadex-G75 column equilibrated with 0.05 M phosphate buffer, $\text{pH } 7.5$, 0.25% gelatin, and eluted with the same buffer. The iodinated product (initial specific activity $\sim 16.5 \mu\text{Ci } \mu\text{g}^{-1}$) was stored at 4°C . Antibodies against ovalbumin were raised in rabbits by injection of 1 mg of ovalbumin (purified by preparative gel electrophoresis) emulsified in Freund's complete adjuvant. A second injection was made 3 weeks later, and bleedings were made 2 weeks after. Antibodies were purified by $(\text{NH}_4)_2\text{SO}_4$ precipitation and chromatography on CM-DEAE cellulose and Biogel P11 columns⁴⁷. Radioimmunoassays were carried out in a final volume of $300 \mu\text{l}$ in PBSA (phosphate buffer 0.1 M , $\text{pH } 7.5$, $\text{NaCl } 0.15 \text{ M}$, bovine serum albumin 5 mg ml^{-1}). To the desired dilution of bacterial extract (or of unlabelled ovalbumin) in PBSA, were added $20 \mu\text{l}$ of mixture containing $15 \mu\text{l}$ of nonspecific rabbit antiserum as a carrier and $10,000 \text{ c.p.m.}$ iodinated ovalbumin. This was followed by addition of $50 \mu\text{l}$ of a dilution of the specific antibodies able to precipitate 50% of the iodinated protein. After an 18-h incubation at 4°C , antibody-bound ovalbumin was separated from free ovalbumin by addition of Na_2SO_4 18% (w/v) final. The precipitate was centrifuged for 5 min at room temperature in a microfuge; supernatants were pipetted and pellets and supernatants counted in a gamma counter. Control experiments with iodinated antibodies demonstrated that precipitation by Na_2SO_4 was essentially complete. Precipitation of iodinated ovalbumin in the absence of specific antibodies did not exceed $5\text{--}8\%$ of input. Strain 1442 (BMH 7156 $\Delta(\text{pro} \text{ lac}) \text{ F}'\text{lac}^{\text{O}^+ \text{Z}} \text{u118}$ was transformed by pOMP2, and grown on glucose minimum plates (to prevent loss of the episome ensuring the overproduction of *lac* repressor). Liquid cultures of 1442 (pOMP2) and C600r $^+$ m $^+$ (pOMP2) in L broth with $5 \mu\text{g ml}^{-1}$ ampicillin were inoculated from plates at about 10^8 cells per ml and grown to about 1.2×10^9 bacteria per ml in the presence or absence of IPTG (5 or 10 mM). In some experiments cultures were directly frozen and sonicated in the presence of protease inhibitor, PTSE⁴⁶ *p*-toluyl-sulphonyl-fluoride, Ega-Chemie, $30 \mu\text{g ml}^{-1}$). In the experiment shown in *a*, bacteria were concentrated 20-fold in PBSA containing PTSE, frozen, sonicated ($2 \times 20 \text{ s}$ in the cold) and diluted serially in the same buffer mixed with a constant amount of extract of the parent strain 1441. Radioimmunoassays were carried out in duplicate. B/B_0 is the ratio between the c.p.m. precipitated in the presence and the absence of bacterial extracts or unlabelled ovalbumin (nonspecifically precipitated c.p.m. being subtracted each time). *a*, Extracts of 1442 (pOMP2) with (▲) and without IPTG (●). The abscissa has been recalculated as μl of nonconcentrated extract ($1 \mu\text{l} = 1.2 \times 10^6$ bacteria). *b*, Standard curve with ovalbumin (Sigma, 99% pure). 50% competition corresponds to 15 ng of pure ovalbumin.

Fig. 6 Size of the ovalbumin-like product in immunoprecipitated bacterial extracts. 5-ml cultures of 1442(pOMP0) (lanes 1 and 2) and 1442(pOMP2) (lanes 3–6) were inoculated at 37 °C in 63B1 glycerol medium supplemented with 5 $\mu\text{g ml}^{-1}$ ampicillin and eventually (lanes 5 and 6) with 1 mM IPTG, grown to an A_{600} of 0.5, labelled with ^{35}S -methionine (Amersham, 50 $\mu\text{Ci ml}^{-1}$, 800 Ci mMol^{-1}) for 30 min and poured over an equal volume of a crushed frozen solution containing 10 mM NaN_3 , 200 $\mu\text{g ml}^{-1}$ chloramphenicol and 30 $\mu\text{g ml}^{-1}$ PTSE. The cells were then centrifuged (5,000 g, 10 min at 4 °C) and frozen at –20 °C. The pellet was resuspended on 0.5 ml of 10 mM Na phosphate buffer, pH 6.8, and sonicated (2 $\times$ 20 s in ice). Immunoprecipitates were prepared as described by Rhoads *et al.*⁴⁷ with minor modifications. To 10⁷ trichloroacetic acid-precipitable c.p.m. were added either 5 μg of unlabelled carrier ovalbumin and 50 μg of bovine serum albumin (BSA) (lanes 3 and 5), or 50 μg of competing unlabelled ovalbumin and 5 μg of BSA (lanes 2, 4 and 6). Samples were adjusted to 1.4% Triton X-100, 1.4% sodium deoxycholate, 10 mM Na phosphate buffer, pH 7.5, and 0.15 M NaCl, to which 20 μl of a nonspecific rabbit serum were added. The mixture (210 μl) was incubated for 30 min at room temperature, layered over 200 μl of 1 M sucrose, 1% Triton X-100, 1% deoxycholate, 10 mM Na phosphate buffer, pH 7.5, and 0.15 M NaCl, and centrifuged (5 min at 4 °C in a microfuge). The upper phase was carefully pipetted and 20 μl of rabbit anti-ovalbumin antibodies, enough to precipitate 7.5 μg of ovalbumin, were added. After 60 min at room temperature, the mixture was layered over sucrose as described above, centrifuged and frozen in liquid N_2 . A 3-mm long tip was cut from the bottom of the tube and the immunoprecipitate was resuspended in 20 μl of 10% glycerol, 2% sodium dodecyl sulphate, 62.5 mM Tris-HCl, pH 6.8, 0.7 M β -mercaptoethanol and bromophenol blue, heated for 2 min at 100 °C and loaded on a sodium dodecyl sulphate polyacrylamide gel. The resolving gel was a 6–15% linear gradient of acrylamide⁴⁸. The electrophoresis was carried out with the apparatus described by Studier⁴⁹ at 4 °C for 12 h at 5 V. After the run, the gel was fixed and stained with Coomassie brilliant blue R-250 (0.2% (w/v) in methanol:water:acetic acid, 50:50:7 v/v), impregnated with diphenylloxazole, dried and fluorographed with flash-activated Fuji RX films according to the standard technique⁵⁰. Exposure was for 24 h at –70 °C. Arrows indicate the position of unlabelled size markers added to each sample: bovine serum albumin (BSA, 68,000 daltons), ovalbumin (OV) and trypsin inhibitor (TI, 21,500 daltons). The immunoprecipitates are contaminated by several bacterial proteins. This is probably due to a weak anti-*E. coli* activity of the rabbit antibodies used. Lanes 1 and 2, 1442(pOMP0); lanes 3 and 4, 1442(pOMP2) without addition of IPTG; lanes 5 and 6, 1442(pOMP2) grown in 1 mM IPTG. In lanes 2, 4 and 6, excess unlabelled ovalbumin was added as a competitor.



We could not clearly demonstrate, in C600 (pOMP2), that the synthesis of the ovalbumin-like product is under the control of the *lac* repressor (which is, at least partially, overtitrated by the operators). Because of this, we introduced pOMP2 into a strain (1442) which overproduces *lac* repressor. Synthesis was then stimulated by the *lac* inducer IPTG about 50-fold (Fig. 5a).

The size of the ovalbumin-like product was estimated as follows: extracts of cells harbouring pOMP2 and labelled *in vivo* with ^{35}S -methionine were precipitated by anti-ovalbumin antibodies, and the dissociated precipitates were loaded on polyacrylamide gels in denaturing conditions as explained in the legend to Fig. 6. The fluorograms in Fig. 6 show a predominant band corresponding to a protein which either co-migrates with or migrates slightly more rapidly than ovalbumin, depending on electrophoresis conditions. In strain 1442 (pOMP2), synthesis of this protein depends on the addition of IPTG. Addition of excess unlabelled ovalbumin in the immunoprecipitation eliminates most of the radioactive band, which indicates that it corresponds to an ovalbumin-like protein. In total labelled extracts not subjected to immunoprecipitation, the band could sometimes be directly observed. In summary, the ovalbumin-like product has a size close to that of ovalbumin.

Synthesis of foreign polypeptides by *E. coli*

We have constructed *E. coli* strains capable of synthesising an ovalbumin-like product. The available evidence indicates that: (1) synthesis of this product is under *lac* repressor control; (2) the product competes efficiently with iodinated ovalbumin in a radioimmunoassay; (3) the size of the product is very similar to that of ovalbumin.

Together, these data strongly suggest that our *E. coli* strains synthesise an ovalbumin-like product, the composition of which is probably very close to that expected, that is, eight amino acids of β -galactosidase and 381 amino acids of ovalbumin (out of the 386 of native ovalbumin). The exact structure of the hybrid protein remains to be investigated.

Bacteria harbouring the recombinant plasmids synthesise an average of 30,000 or 60,000, and up to 45,000 or 90,000 molecules per cell (depending on the interpretation of the radioimmunoassay competition curves). This corresponds to about 0.5–1% of the total protein mass of an *E. coli* cell. These figures are relatively close to the theoretical yield which can be estimated (from the number of β -galactosidase monomers synthesised in cells harbouring a multicopy plasmid with a functional *Z* gene) to be of the order of $1\text{--}2 \times 10^5$ molecules per cell.

The production of high quantities of an ovalbumin-like protein by *E. coli* strains raises several interesting points. First, the strains are perfectly stable and show no tendency to lose the recombinant plasmid. Second, ovalbumin is a secreted protein; in preliminary experiments, we find no trace of ovalbumin-like product in the supernatant of C600 (pOMP2) cultures. Third, the ovalbumin-like protein made by *E. coli* must be relatively stable in the bacterial environment, but no precise measurement has yet been made. Fourth, the codons in ovalbumin mRNA are not randomly distributed²⁶ and their frequency may differ significantly from the frequency of codons in *E. coli*. Some codons (AUC, GUG, CCA, GAC, GAA, GCA) are used more frequently in ovalbumin than in coliphage genes of known sequence^{36,37}, such as MS2 or ΦX174 . The possible deficiency of *E. coli* in the corresponding minor tRNAs does not seem to

interfere much with expression of the ovalbumin gene sequence.

This is, to our knowledge, the first example of synthesis by *E. coli* cells, of a long polypeptide chain from a higher eukaryote. The accuracy of the genetic construction which we made is not perfect, as five amino acids of the natural protein are missing and replaced by a few others from a bacterial protein. There is no theoretical reason, in our opinion, that the site of the junction between the *lac* and the ovalbumin sequences could not be moved down to the ATG initiator codon, leading to the synthesis of an accurate product. This is also the first example of a long polypeptide chain synthesised in good yield from a cloned complementary DNA made from mRNA. As a whole, our experiments demonstrate the feasibility of engineering bacteria for a potentially very large number of uses. Because of its well balanced amino acid content, ovalbumin is often used as a standard for nutritional value, and it is therefore conceivable that the ovalbumin-like product made by bacteria could be used for feeding purposes.

Biohazards associated with the experiments described in this publication have been examined previously by the French National Control Committee. The experiments were carried out accordingly in L1B1 conditions³⁸.

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Nucleotide sequence homology at 12 intron-exon junctions in the chick ovalbumin gene

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A short partial sequence homology is present at all intron-exon junctions, or splice points, in the chick ovalbumin gene; it is probably a signal for a splicing enzyme. The significance of the junction sequences for splicing is discussed. We find no evidence of strong Watson-Crick base pairing between adjacent junctions.

OUR knowledge of the organisation of the ovalbumin gene has advanced rapidly because of the successful cloning of a major part of this gene¹⁻³. Analysis of the clones¹ has shown that the gene is arranged in at least eight separate regions (I-VIII, Fig. 1), which are colinear with the mRNA sequence⁴. But these expressed pieces or exons⁵ are separated by seven sections of

DNA or introns labelled A-G which are absent in the mature mRNA. Given this remarkably complex organisation, how is the mRNA synthesised. Potentially an RNA polymerase could transcribe only the exons leaving the introns alone. This 'jumping polymerase' model⁶ would essentially join up sections I-VIII to give the correct mature mRNA. Alternatively the RNA polymerase could synthesise a copy of the entire gene copying both intron and exon regions. This pre-mRNA could be cut and the exons joined in the correct order, the introns being eliminated. The latter hypothesis seems more likely as there is evidence of a 40S pre-mRNA⁷ containing both exon and intron sequences. This mechanism also occurs for other spliced genes such as those present in adenovirus⁸⁻¹⁰, the β -globin¹¹ and yeast tRNA genes^{12,13}.

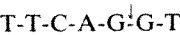
The splicing must be a precise process. It has been suggested that two adjacent exons distant in an immunoglobulin λ chain

three of the four bases and three (A/II, IV/D and V/E) have only two bases in common. If the averaging is extended, a less specific sequence T-T-C-A-G-G-T emerges (Table 1). Further analysis of the nucleotide frequencies on the 5' side shows that T residues are preferred and this is particularly noticeable at the 3' end of introns. An impressive example is junction F/VII (Figs 2*b* and 3) where a 'run' of 16 T residues occurs. Thus T residues are common in the intron sequence immediately preceding (5' of) a junction.

Splice points in the mRNA

Figure 4 shows the accurate location of the seven splice points superimposed on the ovalbumin mRNA sequence⁴. This confirms the previous conclusion that the gene is split into eight pieces¹ and that the splice points are all located in the 5' half of the mRNA. The 'limits' of the individual splice points are indicated, the uncertainty arising from the terminal redundancy at the ends of each intron (see above). For splice points (1) and (7) we do not know whether a similar redundancy exists as the complete intron sequence was not included within the 2.4 and 1.8 kilobase DNA fragments studied here.

It would seem likely, however, that splicing should occur at a unique position within the homologous sequence. We can speculate that it occurs at the arrowed position:



as only this position is common to all the redundant sequences (Fig. 3). Kourilsky and Chambon²⁵ have extended this argument and we can formulate the following simple rule. The first cut by the splicing enzyme occurs immediately 5' to a G-T doublet in an intron, and the second cut immediately 3' to an A-G doublet at the other end of the same intron. This rule works without exception for all the known ovalbumin splice points as well as for the immunoglobulin light chain (see Fig. 3).

The separate exon regions vary from the smallest 49±4 residues in length between splice points (2) and (3), to the longest extending 1,030 residues from splice point (7) to the end of the mRNA. Splice (1) occurs in the 5' non-coding region of the mRNA clearly indicating that residues 1-45 of the mRNA have some important function to perform. Possibly they are needed because they form a highly stable hairpin loop near the cap⁴ which is needed for efficient initiation of translation. An analogous splicing of a 'leader' sequence occurs in adenovirus⁸⁻¹⁰ and SV40 DNA^{15,16}.

Significance of the 'consensus' sequence C-A-G-G

Part of the explanation of the consensus sequence is probably the A-G and G-T doublets defining the splice point (see above). However these doublets alone would not give the observed extensive homology. Presumably this homology is part of the recognition sequence for a splicing enzyme but it is not sufficiently conserved, nor long enough to be a unique signal for a splicing enzyme or enzymes. For example, in the 50% (3,400 residues) of the entire ovalbumin gene that we have sequenced (G.G.B. unpublished results), we find 153 positions of which a 3 out of 4 match for this consensus sequence occurs¹⁶. C-A-G-G occurs at 13 of these points and only three of these positions are splice points. Nevertheless the consensus sequence is well conserved among the 12 splice points in the chick ovalbumin gene when compared with the other genes (immunoglobulin, λ and β-globin) in mice, providing strong evidence that it is conserved in evolution. But it does not occur in the spliced tRNA genes in yeast although they may have their own special mechanism as the introns are very short. The basic homology at all the splice points (excepting the tRNA genes) suggests that we are dealing with a single splicing enzyme or at least a small number of closely related enzymes, with a wide specificity amongst different genes. The alternative view that the homology results from the convergent evolution of many different splicing enzymes—for example, one for each splice point—seems unlikely as there is no logical reason why a common sequence

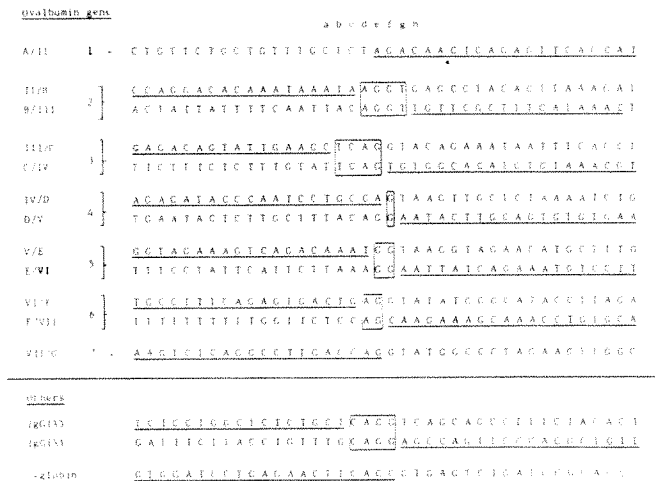


Fig. 3 Sequence at the 12 intron-exon junctions in the ovalbumin gene compared with three other junctions in other genes. For the nomenclature of the ovalbumin junctions see Figs 1 and 4. The terminally redundant sequences (boxed) at each end of an intron were vertically aligned to generate the correct mRNA sequence (Fig. 4) and to maximise homology. Exons are underlined.

should arise. We would expect in that case many different, but unique sequences as occurs for example in the recognition sequence of the many different restriction enzymes¹⁸. We cannot use this homology to definitively predict splice points as the consensus sequence is not characteristic enough. Presumably the additional features, such as the high T content at the 3' end of introns (although a computer analysis shows that this property is not restricted to these regions) and other unknown features allow specific recognition of authentic junctions whilst avoiding other regions.

No obvious base-pairing between adjacent intron-exon junctions

The splicing enzyme needs to do more than recognise specific junction regions. We presume that it must cut and join the correct number of exons in the correct order. Where there are so many (eight) pieces to be joined in a specific order, it would seem extremely wasteful to allow ligation to occur by passive alignment of the correct ends by random diffusion (analogous to the blunt-ended ligation of DNA by the bacteriophage T4 ligase). We favour the hypothesis that a specific mechanism exists to bring the distant exon sequences into close contact. The most obvious hypothesis⁶ is that base-pairing occurs between adjacent junctions, thus generating a large hairpin loop out of the intron. A computer search was made for all secondary structures¹⁹ in Fig. 3 (allowing G-C, A-T and G-T base pairs) that would approximate adjacent junctions. But the free energies (Δ*G*) of the best structures obtained were never less than a few kilocalories²⁰. In every case a more favourable base pairing was possible with known sequences (G.G.B. unpublished) elsewhere in the gene. Despite our inability here to find any positive evidence for a secondary structure model relying on base pairing, we cannot eliminate this mechanism. It is possible that intrinsically quite unstable structures in the pre-mRNA are stabilised by other (tertiary) interactions. For example, there may be long range interactions with other regions in the pre-mRNA or even with other RNA molecules⁶. A more definite analysis of this may be possible when the entire structure of the ovalbumin gene is known. We have also considered models based on the specific affinity of the splicing enzyme(s), or subunits of this, for one another. But such models become attractive only if there are specific and different enzymes for each splice point, as only then could correct splicing be ensured.

Conclusions

The sequence analysis of the 12 intron-exon junctions in the chick ovalbumin gene shows quite clearly that a consensus sequence is present at the splice point. But this homology is only

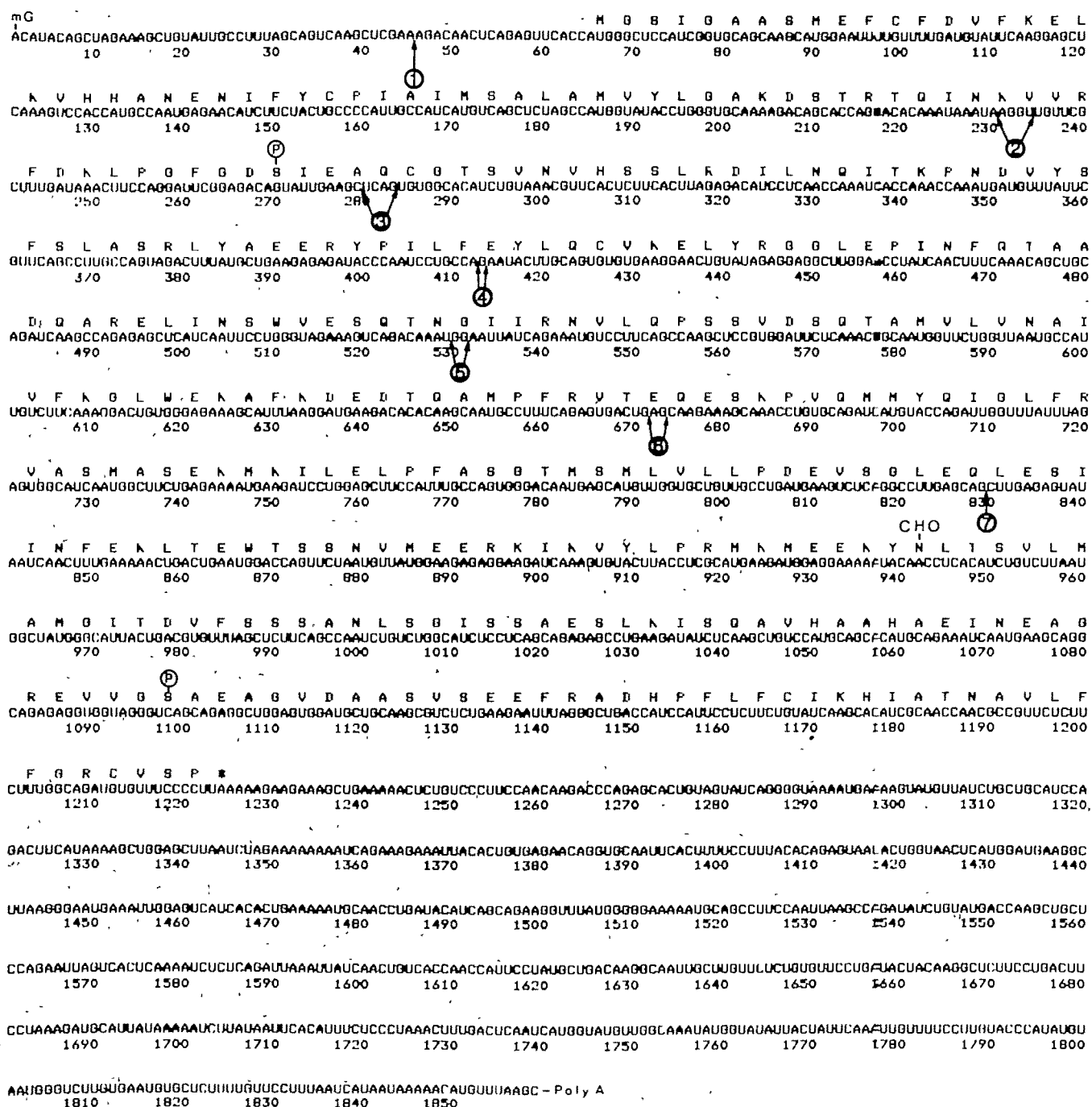


Fig. 4 The seven splice points in ovalbumin mRNA indicated by symbols ①-⑦. In no case is the splice point precisely known because of terminal redundancy at the ends of the respective intron regions.

partial. This is reminiscent of sequence studies of other important controlling regions such as the promoters in DNAs²¹, or ribosome binding sites in messenger RNA²². Unfortunately the sequence analysis has not elucidated the mechanism by which adjacent exon regions are brought into close contact for splicing. It does seem unlikely that strong Watson-Crick base pairing between adjacent junctions occurs, although we cannot eliminate weak interactions stabilised by other factors. Clearly a direct study of the enzyme or enzymes involved is needed for a more complete understanding of this problem²³.

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letters to nature

A larger and more turbulent Universe

THE modern version of the Hubble diagram in which the log of the redshift is plotted against apparent magnitude corrected for redshift and absorption effects of clusters of galaxies, is in principle capable of yielding several cosmologically useful pieces of information. In this letter I report evidence for a significant and substantial departure from the expected redshift for the Virgo cluster of galaxies. A peculiar velocity of $658 \pm 96 \text{ km s}^{-1}$ is derived but because of Virgo's membership in the Local Supercluster, such a value is not unexpected. Thus the turbulence recently observed by Smoot *et al.*¹ for the Galaxy's motion within the Hubble expansion is present elsewhere in the Universe as well. Because the Virgo cluster does not exactly follow the Hubble law, a new value for the Hubble constant equal to $43.4 \pm 2.6 \text{ km s}^{-1} \text{ Mpc}^{-1}$ is found leading to an observable Universe 1/4 to 1/3 again as large as was previously recognised.

When objects with reasonably consistent and sufficiently large luminosities, such as members of clusters of galaxies, are plotted in the Hubble diagram, the mean relation will have the theoretical slope of 1/5 and a dispersion, which because of the nature of a log plot, is greater at low redshifts. The redshift residual of a cluster, if sufficiently greater than the standard deviation about the mean relation, gives the cluster's peculiar radial velocity. If the absolute magnitude of a cluster object is known, its apparent magnitude and the redshift taken from the mean cluster relationship yield the Hubble constant, H_0 . In addition, the curvature of the mean relationship at very large redshifts can give information on galaxy luminosity evolution and the deceleration parameter.

Table 1 Data for clusters of galaxies

Cluster	$c\Delta\lambda/\lambda_0$ (km s^{-1})	Δv (km s^{-1})	$\log cz$	V^*	K_v	A_v
Virgo	$1,144 \pm 68^{6,7}$	361 ± 55	3.1782	10.0^{16}	0.01	0.01
A1367	$6,424 \pm 238^8$	371 ± 57	3.8328	14.32^4	0.04	0.01
A1656	$6,943 \pm 61^9$	315 ± 48	3.8614	14.7^{17}	0.05	0.00
A2199	$9,002 \pm 180^{10-13}$	66 ± 10	3.9577	15.4^{17}	0.06	0.09
A151	$15,749 \pm 309^6$	-328 ± 50	4.1876	16.4^{17}	0.08	0.00
A754	$16,256 \pm 205$	331 ± 51	4.2202	16.78^4	0.08	0.26
A2065	$21,384$	161 ± 25	4.3336	$17.10^{4,17}$	0.10	0.04
	$\pm 298^{6,14*}$					
A2670	$22,491 \pm 300^{15}$	-379 ± 58	4.3441	17.3^{18}	0.11	0.01

The first column gives the cluster identity, the second the redshift in km s^{-1} referred to the Sun, the third the velocity correction of Smoot *et al.* in km s^{-1} , the fourth the log of the relativistically combined redshifts, the fifth the inflection point visual magnitude, V^* , the sixth the K -correction from Schild and Oke⁵, and the seventh the galactic absorption computed from $A_v = 0.19 (\text{csc} |b| - 1)$. The redshift of cluster A754 is an average of three unpublished redshifts which I obtained with the image tube spectrograph and 2.1-m telescope of Kitt Peak National Observatory in February 1975 at a reciprocal dispersion of $111 \text{ \AA mm}^{-1}$. The data represent the combined results of several thousand galaxy magnitude measurements and several hundred galaxy redshift measurements.

* Because of a calibration error in ref. 14, all redshifts were decreased by 121 km s^{-1} .

Although we are far from learning a great deal about evolution and the deceleration parameter from the Hubble diagram, it is now possible to say something about H_0 and the peculiar velocity of at least one of the nearer clusters. This has come about through the use of a reasonably accurate way to gauge relative cluster distances and through a better knowledge of the cluster redshifts. Whereas use of the brightness of the first-ranked cluster galaxy as a distance indicator yields a standard deviation of 0.30 magnitudes about the mean Hubble relation², use of the inflection point in the cluster luminosity function yields a standard deviation almost 3 times smaller^{3,4}. Following the detection of an anisotropy in the cosmic blackbody radiation¹, explained as solar motion of $390 \pm 60 \text{ km s}^{-1}$ relative to the average Hubble expansion in the direction of right ascension and declination, $\alpha = 1 \text{ h}$, $\delta = 6^\circ$ (or galactic longitude and latitude, $l'' = 248^\circ$, $b'' = 56^\circ$), it becomes possible to correct the observed cluster redshifts. For distant clusters this correction is fractionally small but for the nearest ones, proportionally larger changes occur. For the Virgo cluster in particular, the derived peculiar velocity is found to be significantly larger.

Table 1 presents the data for eight clusters. The straight line in Fig. 1 is the best fit with the theoretical slope 1/5 to the 7 most distant clusters and has an intercept on the $\log cz$ axis of 0.9319 ± 0.0091 . Such a fit must be made only to data points with redshifts large enough for the effects of any peculiar velocities to be proportionally small. Because Virgo's corrected redshift is only $1,507 \text{ km s}^{-1}$ and its derived peculiar velocity is almost half this value, the Virgo point must be excluded from the fit. For the most distant cluster (A2670), a peculiar velocity equal to that of Virgo's would be only 3% of the corrected redshift and would result in an additional residual about the mean line of only 0.0137 in $\log cz$ or 0.07 mag.

The standard deviation for the fit of the seven most distant clusters about the straight line is only 0.12 mag. This dispersion is by far the smallest of any of the methods of gauging relative cluster distances. The cosmic dispersion of M_v^* must be even smaller than this value because the precision in determining V^* from the luminosity functions is at present about $\pm 0.05 \text{ mag}$, the galactic absorption is most likely 'spotty', the cluster redshifts have a non-zero mean error, and the clusters other than Virgo may have peculiar radial velocities as well. A trial fit excluding also the second nearest cluster (A1367) reduces the standard deviation to only 0.07 mag.

The Virgo cluster point lies 1.25 mag to the left of the mean relation for the seven clusters but because the residual is greater than 10σ , the Virgo cluster is interpreted as having a peculiar radial velocity of 658 km s^{-1} . The various sources of uncertainty give the standard deviation of this value as 96 km s^{-1} ; thus the Virgo peculiar velocity is certain at almost the 7σ level. An earlier preliminary calculation without the velocity correction gave a peculiar velocity about one half as large¹⁹. This result contradicts the assertions of Sandage and Tammann²⁰ who discount any peculiar velocity for Virgo and argue that the local velocity field is as quiet as it can be²¹.

The Virgo peculiar velocity indicates velocity irregularities on a scale size of about 20 Mpc. Such a condition is not unexpected because the Virgo cluster is a member of the Local Supercluster and must move within it. Following Abell¹⁷, for a supercluster of this size in equilibrium, with a mass of 10^{16} solar masses, and an isotropic velocity field, a radial velocity dispersion of about

850 km s⁻¹ is predicted. The peculiar velocities exhibited by the Galaxy (including its neighbours in the Local Group) and the Virgo cluster may be examples of the motions of clusters within bound superclusters and therefore manifestations of a universal turbulence existing since the early stages of the Universe.

If on the other hand the Local Supercluster is not bound, a cluster with a large peculiar velocity will eventually catch up to clusters with the average Hubble expansion and thus the peculiar velocity will die out with time. In the past the peculiar velocities of clusters would have been even larger. A third possibility, suggested by Rowan-Robinson²², is that entire superclusters are in rapid motion relative to each other. As superclusters are not known to be bound to each other, this motion would also die out with time and would also have been greater in the past. This third situation is not inconsistent with either of the first two—although the first and second are incompatible with each other—and we may in fact be experiencing some combination of motions.

An apparent inconsistency between the solar motion of Smoot *et al.*¹ and that of Rubin *et al.*^{23,24} deserves comment here. From a study of 96 galaxies with 3,500 < *cz* < 6,500 km s⁻¹ Rubin *et al.* derive a solar motion of 600 ± 125 km s⁻¹, but in a direction about 109° away from that of Smoot *et al.* However if Rubin *et al.*'s sample of galaxies has a peculiar velocity of 815 km s⁻¹ relative to the average Hubble flow and in a direction toward α = 12.5 h, δ = 33° (*l*^{II} = 298°, *b*^{II} = 30°) the discrepancy is explained. The picture that is emerging at present therefore is of a Universe that is much more turbulent than previously thought. The quiet velocity field of Sandage and Tammann then is explained by the absence of a correction for the Galaxy's peculiar motion and a less accurate distance indicator (their results yield 34 km s⁻¹ for the Virgo peculiar velocity).

The Hubble constant is derived from the mean cluster predicted redshift of 847 ± 38 km s⁻¹ for Virgo and its distance in Mpc's. Table 2 lists several distances for Virgo taken from the literature. Because the Virgo peculiar velocity was not previously known, the Hubble constants derived here are

Fig. 1 The Hubble diagram for cluster of galaxies based on the inflection point magnitude, *V*^{*}, as a distance indicator. For the best fit to the seven most distant clusters with the theoretical slope of 1/5, the standard deviation is only 0.12 mag. The error bars given the mean errors of the corrected redshifts. Except for the two nearest clusters the mean errors are too small to be shown. The large residual for the Virgo cluster point at the lower left indicates a substantial peculiar velocity for this cluster.

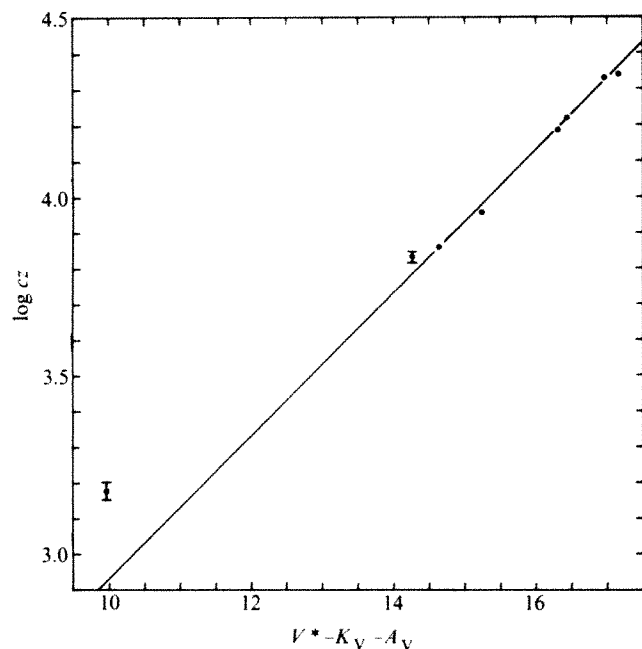


Table 2 Distance of the Virgo cluster and the Hubble constant

Reference for distance	Corrected (<i>m</i> - <i>M</i>) _{AB}	(<i>m</i> - <i>M</i>) _T	<i>D</i> (Mpc)	<i>H</i> ₀ (km s ⁻¹ Mpc ⁻¹)
25, 26	30.9 ± 0.2	30.62 ± 0.2	13.3 ± 1.2	64 ± 7
27, 26	31.3 ± 0.5	31.02 ± 0.5	16.0 ± 3.7	53 ± 13
28, 26	31.9 ± 0.4	31.62 ± 0.4	21.1 ± 3.9	40 ± 8
20	—	31.45 ± 0.09	19.5 ± 0.8	43.4 ± 2.6

Column one gives the reference, two the apparent blue distance modulus corrected as described below, three the true distance modulus, four the distance in Mpc's, and five the Hubble constant in km s⁻¹ Mpc⁻¹. Racine's²⁵ apparent blue modulus has been increased by 0.2 mag to account for a recalibration of his photographic B magnitudes given by Ables *et al.*²⁶. Racine's distance is based on a comparison of globular cluster brightnesses in M87 with those in M31 and the Galaxy. Because the Galaxy is deficient in globular clusters this distance may be too small. Sandage²⁷ and van den Bergh²⁸ compared globular cluster brightnesses in M87 with those in M31 alone. Since their results are based on Racine's measurements however, their moduli have been increased by 0.2 mag also. For these three values the true distance modulus was obtained by subtracting 0.28 mag to account for galactic absorption and *K*-dimming (0.26 and 0.02 mag, respectively). Sandage and Tammann²⁰ give only a true distance modulus based on the brightnesses of Virgo spirals, with the spiral calibration being based on the diameters of HII regions.

significantly smaller than those derived previously. Racine had reported *H*₀ = 79 ± 12, Sandage 75.3 ± 17, van den Bergh 60 ± 12, and Sandage and Tammann a local value of 57 ± 3 and a global value of 55 ± 6 km s⁻¹ Mpc⁻¹. The new values of the Hubble constant in Table 2 are smaller by 19–33%.

On the basis of the least formal error in the distance modulus, the best estimate for the Hubble constant is *H*₀ = 43.4 ± 2.6 km s⁻¹ Mpc⁻¹ which leads to an observable Universe about 1/4 to 1/3 larger than was previously recognised. The corresponding Hubble time (time since the beginning of the Universe if there was no slowing of the expansion) is 22.5 ± 1.4 × 10⁹ yr. As the oldest known stars are about 15 × 10⁹ yr old, there may have been some slowing of the expansion and/or a delay of star formation, possibly due to the turbulence.

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Relativistic jets and beams in radio galaxies

RADIO-ASTRONOMICAL observations have recently clarified the link between the components of extended double sources and the primary power supply in the central galactic nucleus. The new data vindicate the general idea¹⁻⁴ that power is continuously supplied by beams; it seems, furthermore, that the beams are collimated in a scale little larger than the central power supply ($\ll 1$ pc), and that the orientation remains fairly steady over the whole lifetime. The giant double source 3C236, 2×10^7 light yr in total extent^{5,6}, has a compact central component aligned with the overall axis⁷; a similar phenomenon is observed in Cygnus A (ref. 8). In NGC6251, a straight jet 200 kpc long⁹ emanates from a 'blowtorch' ≤ 0.1 pc wide in the galactic nucleus¹⁰. There is a radio jet¹¹ in 3C147 reminiscent of the well-known features in M87 and 3C273; and very long baseline interferometry (VLBI) reveals linear structure in several compact extragalactic sources¹². It is argued here that collimation occurs close to a central collapsed object, and that the beams are orientated along its spin axis. Strong-field gravitational effects then stabilise the beams against jitter even if the gas fuelling the source has an inconstant flow pattern. Radio galaxies where the beam axis seems to have gradually drifted or swung, rather than pointing in a constant direction, may belong to a special class that have experienced collisions and recurrent nuclear activity.

The beams squirt from active nuclei with velocities approaching c . The most direct indications of relativistic bulk motions are the 'superluminal' variations¹² in compact sources: the detailed kinematics are still obscure—from a surfeit¹³, not an absence, of acceptable models—but most ideas entail bulk plasma motions at speeds $\sim c$. Indirect evidence for collimated relativistic outflow comes from the polarised optical continuum outbursts in objects such as A0 0235+164 (ref. 14), which pose severe interpretative problems unless the emitting material is directed towards us at speed $\sim c$ (ref. 15). Doppler-favouritism of the approaching beam, when speeds are relativistic, yields a natural explanation for the oneness of jets in, for instance, M87 (refs 16–18).

The central 'engine'—already inferred from source energetics to be highly efficient—seems therefore able to generate plasma whose mean kinetic energy per particle approaches the rest mass energy. Prime candidates for efficient engines are black holes of $\sim 10^8 M_\odot$ accreting from their surroundings: efficiencies can then attain $\sim 10\%$, and the output can persist for the whole $\sim 10^8$ yr lifetime of a giant source like 3C236.

Many variants of this scheme have been theoretically investigated, and almost all can generate the required high mean energy per particle^{19–27}. This is basically because the energy release is concentrated within a few Schwarzschild radii ($r_s \approx 3 \times 10^{13} (M/10^8 M_\odot)$ cm) where the keplerian speeds are $\sim c/2$, as is the sound (or Alfvén) speed in pressure-supported gas. Possible processes near the hole may involve high ion temperatures^{22,27}, relativistic shocks, radiation pressure, magnetic flares^{23,24}, or pulsar-type electromagnetic processes^{25–27}; but all options suggest that a relativistic electron-ion (or e^+e^-) plasma may be the primary product of the gravitational energy released by captured material.

Collimation of this relativistic plasma must demand some asymmetry in the surroundings, probably due to rotation. For instance, if the central power-source is embedded in a rotationally flattened cloud, the plasma will tunnel its way out by excavating nozzles along the rotation axis. This 'twin exhaust' configuration³ (which can create directed beams even if the plasma is generated with an isotropic pressure) could be established on any scale: in particular, there is no reason that the nozzles should not be at $\leq 100 r_s$ if the outflowing plasma originates very near the hole.

The nozzle walls could be confined by hot inwards-spiralling material forming a thick disk or 'donut', centrifugally inhibited from draining into the hole^{29–32}. This confining material would be supported by the pressure of radiation, ions, or magnetic fields. The shape of the nozzles depends on the precise pressure distribution and rotation of the confining cloud, but the walls lie outside the (roughly paraboloidal) surface within which a particle would have so little angular momentum that the hole could swallow it.

Collimation mechanisms influenced by non-newtonian gravitational effects near the hole have some peculiarly interesting consequences, discussed below. Such influences are restricted to length scales much smaller than those for which we have direct radio evidence. The beams, as they propagate outwards, cool in accordance with Bernoulli's equation; they may also cool radiatively, the resulting emission perhaps representing the non-thermal optical continuum from variable galactic nuclei¹⁵. Re-randomisation of bulk kinetic energy occurs in the components of extended double sources where the beams impact on an external medium³. Radiation from the beams themselves (and the superluminal compact sources in particular) indicates some dissipation along the beam due to shock formation, entrainment, or friction. The beam speeds may well remain relativistic out to distances of hundreds of kpc, at least in the stronger sources, otherwise the residual power input into the extended components would be much less than that dissipated by friction along the beam.

The common statement that the beams should be aligned with the associated galaxy's rotation axis (minor axis?) deserves more analysis. One should distinguish the rotation axes of the galaxy, of the infalling gas (which provides the fuel and influences the collimation), and of the black hole itself; anisotropies in the extragalactic environment may complicate the picture still more.

If the relevant gas comes from general stellar mass loss, and pervades (or has been accreted from) the whole galaxy, then its angular momentum vector should coincide with the galaxy's. The same statement holds for primordial gas falling into an elliptical: after one or two orbits its distribution would have become axisymmetric with respect to the galaxy³³. Thus, barring such complications as triaxiality³⁴, the gas should provide a stable long-term symmetry axis. But this gas may not provide an adequate fuelling rate for the most powerful sources³⁵ (unless it accumulates and is then dumped sporadically). This suggests that there may be an extra supply of gas near the nucleus. For instance, Hills^{20,21} invokes a 'debris cloud' of disrupted stars, in which the velocity dispersion is $v_* \approx 10^3$ km s⁻¹. The storage time for gas in this cloud is $t_{\text{store}} \approx (r_s/c)(v_*/c)^{-3}$. The number of stars contributing to the cloud at any instant is then $N \approx t_{\text{store}} \dot{M}/m_*$; the angular momentum vector then jitters, on a timescale $\sim t_{\text{store}}$, by $\sim (N\epsilon)^{-1}$ rad, ϵ being the oblateness of the central star cluster. This could be unacceptably large, suggesting the need for some other process to stabilise the beam orientation.

If the black hole is spinning, the Lense-Thirring 'dragging of inertial frames' influences material sufficiently close to it. An orbit of radius r and period t_{orb} that does not lie in the hole's equatorial plane precesses on a timescale $t_{\text{prec}} \approx t_{\text{orb}}(J/J_{\text{max}})^{-1}(r/r_s)^{3/2}$, J being the hole's angular momentum and J_{max} the maximum angular momentum of a Kerr hole. If the material drifts inwards only slowly (that is, $t_{\text{infall}}(r) \gg t_{\text{orb}}(r)$) then the precession builds up over many orbits. As Bardeen and Petterson³⁶ pointed out, there is then a critical radius r_{BP} at which $t_{\text{prec}} \approx t_{\text{infall}}$. For $r \leq r_{\text{BP}}$, the gas flow is axisymmetric with respect to the hole, irrespective of its original angular momentum. Thus, if the beams are collimated at a radius $\leq r_{\text{BP}}$, their orientation is, in effect, stabilised by the hole; they are then impervious to jitter resulting from short-term (timescale $\ll M/\dot{M}$) fluctuations in the flow pattern of the surrounding gas. If this is the reason for the extraordinary long-term stability of 3C236, the ratio of the size of the source to the dimensions of the nozzle must be $\geq 10^{10}:1$.

Black holes may generally develop from an accumulation of gas with the same provenance as that which later fuels the accretion process. We would then expect alignment of the hole's axis with the angular momentum of the galaxy. But one can envisage circumstances when a galaxy might acquire a misaligned black hole. Suppose, for example, that a galaxy harbouring a central hole—a relic of earlier violent activity—collided or merged with another galaxy³⁷. The new merged galaxy would then be misaligned with its central hole. (The same holds true if the merging galaxies each contained a black hole, and the two holes coalesced.)

What happens if nuclear activity in this merged galaxy is revived by capture of intergalactic gas?³³ Beams collimated at radii $\leq r_{BP}$ will emerge along the hole's rotation axis. But this axis will gradually swing as the accretion proceeds; owing to the Bardeen-Petterson³⁶ effect, each unit mass of infalling material changes the hole's angular momentum by $\sim J_{\max}(r_{BP}/r_s)^{1/2}$. The timescale for the hole to align with its new surroundings is thus $t_{BP} \approx (M/\dot{M})(J/J_{\max})(r_s/r_{BP})^{1/2}$. (This process is more rapid than any forced precession³⁶, unless the black hole acquires a charge which permits electromagnetic torques to act on it³⁸). The value of t_{BP} cannot be estimated precisely, but is of the same general order ($\sim 10^8$ yr) as the overall lifetime of active nuclei.

Reviewers of morphology^{6,39} have noted a class of sources—for example, NGC315, 3C47, 3C192 and perhaps Cygnus A—characterised by reflection symmetry: it seems from the radio contours as though the two symmetrical oppositely-directed beams have gradually swung or drifted over the source lifetime. Perhaps these radio galaxies are systems where a pre-existing central black hole is gradually being torqued into alignment with the angular momentum of newly supplied fuel that has reactivated the nucleus. The gas and plasma dynamics in these sources must be even more complex than in typical doubles. Each beam would be slightly skewed by the transverse drift, the cocoon surrounding it being smeared into a fan shape. (The existence of sharply curved beams in trail sources such as NGC1265 shows that instabilities are not catastrophic even when there is a strong transverse pressure gradient⁴⁰.) This class of sources obviously holds clues to the collimation mechanism and beam dynamics; but they also have added interest if their distinctive morphology is indeed a manifestation of general relativistic effects near a black hole, the scale of the phenomenon amplified 10^{10} -fold by the plasma beams.

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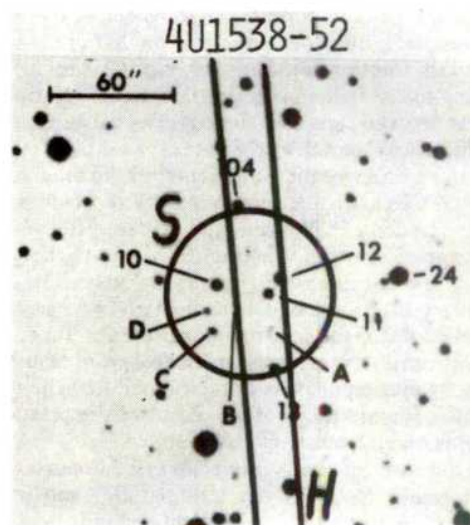
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Location of pulsating binary X-ray source 4U1538-52 with the HEAO-1 scanning modulation collimator

THE 529s pulsating^{1,2} X-ray source 4U1538-52 has been found to be a 3.73d eclipsing binary system³. The most accurate orbital elements yet determined⁴ imply that the primary is an early type giant or supergiant star. Independent determination of the primary mass will make it possible to estimate the mass of the (assumed) neutron star secondary. Cowley *et al.*⁵ have surveyed the stars in the Uhuru¹⁹ and Ariel 5⁴ error boxes and called attention to 10 stars consistent with the inferred properties of the primary. A precise (40") position of 4U1538-52 obtained by SAS 3⁶ includes only two of these stars⁵. This letter reports a HEAO-1 position measurement of precision 12" in one dimension which clearly selects one of these two stars (number 12) as the candidate identification.

The HEAO-1 observing programme allowed the scanning modulation collimator (MC) experiment^{7,8} to observe this region from 20 to 28 August 1977. Three constraints limited the data we could use in determining the position: the necessity to avoid the nearby source MX1608-52, which was in a strong transient state at this time^{9,10}; the lack of data during the X-ray eclipses; and the progressive deterioration and failure of one of the four MC2 (120" FWHM modulation collimator) proportional counters during this period. (The one counter was identified and turned off on 29 August 1977, and did not affect MC2 data subsequent to that date.) We therefore obtain only a

Fig. 1 Circle, SAS 3 90% confidence error region. Heavy lines, boundaries of 90% confidence error region determined by the 30 arc s modulation collimator. Numbered stars were observed by Cowley *et al.*⁵. Lettered stars appear in the finding chart given by Apparaio *et al.*⁶.



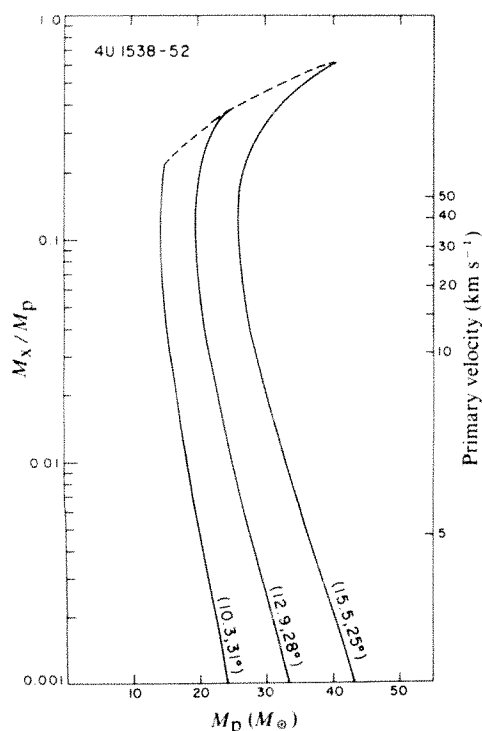


Fig. 2 Abscissa, mass of the primary in units of the solar mass. Ordinate: $q = M_x/M_p$ on left scale, K = orbital velocity of primary on right scale. Dashed curve: upper limit to allowed parameters if the inclination $i = 90^\circ$. The solid curves are denoted by (X-ray mass function, eclipse half-angle) and enclose the region allowed for $f_M = 12.9 \pm 2.6 M_\odot$ and $\theta_e = 28^\circ \pm 3^\circ$.

single line of position, using 12 orbits of data from MC1 (30" FWHM modulation collimator) on 25 August 1977. The half-width of the error region is 12" (90% confidence), considering counting statistics and aspect solution accuracy. We measure an average flux of $(1.2 \pm 0.2) \times 10^{-10} \text{ erg cm}^{-2} \text{ s}^{-1}$ (2–6 keV), with an additional systematic uncertainty of about 30%.

Figure 1 shows this line of position with the SAS 3 error circle. Stars 10 and 12 are among the candidates noted by Cowley *et al.*⁵. Of all the stars having m_v and $B - V$ consistent with an OB supergiant as required by the X-ray mass function^{3,4}, only star 12 is allowed by the present location. Preliminary optical reports indicate that star 12 is an early-type star¹¹ with He I and H α emission¹², and with radial velocity variations $K \sim 25 \text{ km s}^{-1}$ consistent with the X-ray orbital period, thus proving the identification¹³.

The present system is the fifth example of an X-ray pulsator with a well-defined binary period. The transient 4U0115+63 is the sixth example²⁰, but does not show an X-ray eclipse and therefore a mass solution for the X-ray emitter can not yet be made, pending measurement of ellipsoidal light variations. For the other four neutron stars as a class, it can be established that none has a mass less than 0.6 or greater than $4.0 M_\odot$ (refs 14, 15). For 4U1538–52, the measurements⁴ of the mass function give $f(M) = 13 \pm 2.6$ and measurements of the eclipse semi-duration give $\theta_e = 28^\circ \pm 3^\circ$. We can then express¹⁵ the mass ratio $q = M_x/M_p$ of the X-ray emitting (neutron) star to the primary star as a function of the unknown primary star mass (Fig. 2). This approach differs from that of Davison *et al.*⁴ who assumed a range of 1 to $2 M_\odot$ for the neutron star mass and then derived properties of the primary. Here we make *a priori* assumptions on the mass of the compact star. It is assumed that the primary fills its Roche lobe, and the average radius of the primary as a function of q is taken from Kopal's¹⁷ Table 3-2.

From the duration of the X-ray eclipse we deduce that the primary star radius R_1 is greater than $10 R_\odot$, and from the semi-major axis⁴ $a_x \sin i = 55.2 \pm 3.7$ light-seconds we deduce¹⁷

$R_1 \leq 34 R_\odot$. These limits restrict the star to be either an early type (OB) or a red giant. However, red giants have masses $< 10 M_\odot$, and are directly excluded by the mass function value, as shown in Fig. 2.

For the present candidate, Cowley *et al.*⁵ give a measured $B - V = 2.1$ and apparent $V = 14.1$. Since $(B - V)_0 = -0.3$ for an OB supergiant¹⁶, we have $E_{B-V} = 2.4$ and $A_V = 3.2 E_{B-V} = 7.7$. Such a large reddening in this direction ($l = 327^\circ$, $b = 2.2^\circ$) implies a source located at least 2 kpc distant, behind the Sagittarius–Carina spiral arm. The absolute magnitude is then $M_V \leq -5.4$, and the bolometric luminosity $L_{\text{bol}} \geq 2 \times 10^{38} \text{ erg s}^{-1}$. If we equate $L_{\text{bol}} = \pi R_1^2 \sigma T^4$, we obtain effective temperatures of 1.5 to $3 \times 10^4 \text{ K}$, consistent with B spectral type¹², for the above range of primary star sizes.

From Fig. 2 the allowed range of values of the neutron star mass can be determined for any given primary star mass. For a B0 supergiant, we would expect $M_p \sim 35 M_\odot$ (ref. 22). This would imply $M_x/M_p < 5 \times 10^{-3}$, or $M_x < 0.18 M_\odot$. However, stars in close binary systems are typically less massive than indicated by the spectral type^{15,18,23}, and we could clearly have $M_p = 25$, and any $M_x \leq 10$. Crampton *et al.*²¹ have reported an optical velocity of $33 \pm 7 \text{ km s}^{-1}$. This formally gives $0.08 \leq q \leq 0.13$. From the X-ray mass function and eclipse duration as expressed in Fig. 2, we can derive corresponding limits of $1.2 \leq M_x \leq 3.3$. If the optical velocity were precisely 33 km s^{-1} , uncertainty in the X-ray mass function would allow $1.5 \leq M_x \leq 2.7 M_\odot$.

Determination of the masses of large numbers of neutron stars is of the utmost importance to discriminate among the upper limits to the mass allowed by various models of nuclear structure in the framework of general relativity. For the system 4U1538–52 we still need proof that the compact object is indeed a neutron star, for example, by observation of changes in the pulse period which may be attributed to accretion torques. Further optical study of star 12 is clearly needed to confirm the optical observation of the 3.73-d period, and to measure the optical mass function and ellipsoidal light variations as accurately as possible.

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Io may have a bright dawn terminator

NELSON AND HAPKE¹ have shown that occasional observations of post eclipse brightenings (PEB) of the planet Jupiter's satellite Io are very strongly positively correlated with the occurrence of major solar flares within 10° of the solar central meridian (as seen from Jupiter) during the 100 days before the eclipse observation. No eclipses which showed no brightening were preceded by such flares. Io is in the outer part of the jovian radiation belts. Energetic particle fluxes in the Earth's radiation belts at a roughly corresponding location are enhanced for roughly similar times (the 100 days in ref. 1) following the major geomagnetic storms caused by major flares near central meridian^{2,3}. The 100-day time scale is also in reasonable agreement with diffusion times in recent models of the jovian magnetosphere⁴. Considering all this, Nelson and Hapke inferred from their flare correlation that the occurrence of a noticeable PEB effect is due to enhanced fluxes of trapped energetic particles. They suggested that there may be a process similar to thermoluminescence, which converts some fraction of the particle dose received during the eclipse, into visible light which is emitted when the surface is again sunlit, and decays with the characteristic PEB time scale of ~10 min. Although Fanale *et al.*^{5,6} had already suggested that solid state effects in the presence of irradiation by energetic particles might play a part in the PEB phenomenon, the particular correlation with solar flares that Nelson and Hapke found points at once to an energetic particle effect because that is what would be expected to be enhanced by such flares, and at the same time apparently explains the erratic occurrence of PEB on Io and its absence on more distant satellites (because they see much lower fluxes of trapped particles, enhanced or not). In any case, Nelson and Hapke have made a novel yet plausible suggestion which can be tested by laboratory simulation experiments of the solid state process, by more eclipse observations from Earth, and by observations of jovian trapped particles by future flyby and orbiter spacecrafts, and their relation to solar flares. This note suggests another particular test of the thermoluminescence properties of Io which can be made with the unique optical observations which are programmed for the close Voyager I Io flyby in March 1979, and which does not necessarily require a previous suitable flare, as the PEB phenomenon seems to do.

If Io's surface is thermoluminescent with the characteristic PEB time scale of ~10 min, there should be a characteristic bright rim on the sunlit side of Io's dawn terminator. This is essentially because each point on the surface has been 'eclipsed' by Io itself during the night, which is 10 times longer than eclipse, and should accumulate particle dose during the night just as the whole surface would do when eclipsed by the planet. At dawn, it must release all this dose as light on the 10-min time scale. If the time constant in the dark is fairly long compared to the 21-h Io

night, the dawn terminator will be 10 times brighter per unit particle flux than the PEB effect.

To illustrate this, consider a model in which D = stored dose, erg cm^{-2} ; K = light emission rate per stored dose = $K_L \sim 0.1 \text{ min}^{-1}$ in sunlight = K_D ($\ll K_L$) in the dark; e = efficiency of the process; F = particle dose rate, $\text{erg cm}^{-2} \text{ sec}^{-1}$; L = luminosity = KD ; t_e = duration of eclipse. Then, both in sunlight and in the dark,

$$\frac{dD}{dt} = -KD + eF \quad (1)$$

After a few times 10 min in sunlight, the luminosity reaches its equilibrium value $L = eF$. During eclipse or during the night, taking $t = 0$ at immersion

$$L = (K_D/K_L)eF \exp(-K_D t) - eF(1 - \exp(-K_D t)) \quad (2)$$

After eclipse, or at dawn (now taking t as the local Io time since the Sun re-appeared),

$$L = (K_L/K_D - 1)(1 - \exp(-K_D t_e))eF \exp(-K_L t) + eF \quad (3)$$

the spatial shape of the bright dawn terminator in this model is given by replacing t in equation (3) by Φ/Ω , where Φ is longitude from the Io dawn terminator, and Ω is the rotation rate $2\pi/1.79$ days. If $K_D t_e \ll 1$, the luminosity immediately after eclipse or dawn, is

$$L_B \sim (1 + K_L t_e)eF \quad (4)$$

This is about 15 eF for jovian eclipses and about 130 eF for the bright dawn terminator effect. The light curve predicted by equations (2) and (3) with $K_D t_e \ll 0.1$ is shown in Fig. 1.

There are two areas where the idea of bright dawn terminator suggested here is related to the Nelson-Hapke model of PEB. (1) The size and shape of the bright region near the dawn terminator depends in detail on the parameters in equation (3). If one supposes that thermoluminescence occurs on Io without specifying the rate constants, a distinct small bright area is not generally predicted. It occurs in our model because we use the PEB decay rate of 0.1 min^{-1} for K_L . (2) The actual luminosity of the bright area near the terminator is predicted by equation (4) to be 130/15 times the brightness of PEB at the same particle dose rate. Turning this around, one concludes that if it is fairly sure that the dose rate enhancements induced at Io by solar flares are not much more than a factor of 130/15, the bright dawn terminator should be at least as bright as the PEB.

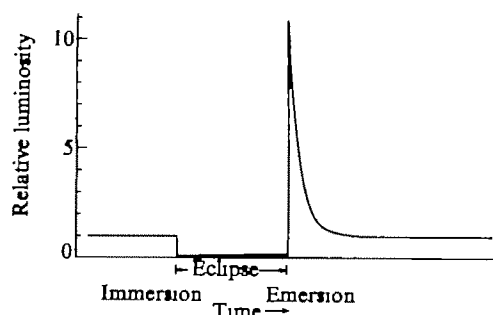
The important point about the predicted bright dawn terminator is that it would provide an opportunity to observe the colour and temperature dependence of the suggested Io thermoluminescence whenever the flyby spacecraft arrive, since it does not require an eclipse and may not require enhanced particle fluxes.

The bright dawn terminator should extend about 10 min of the Io day from the geometrical terminator in our model. This is only $(10 \text{ min}/42 \text{ h}) \times 360^\circ = 1.4^\circ$ of Io longitude into the sunlit side. It is not surprising this has never been observed from Earth, as it is small and highly foreshortened.

On 5 March 1979, Voyager I will observe Io optically from altitudes of 22,000 to 65,000 km, including large areas around both the dawn and dusk terminators within about 60° of Io's south pole, in six spectral regions defined by colour filters from violet to yellow (E. C. Stone, personal communication). The spatial resolution of these pictures should be adequate to reveal the bright dawn terminator effect if it exists and is bright enough.

The shape of the predicted bright area near Io's dawn terminator is actually determined by how K_L (see, for example, ref. 1) really changes locally as the Sun rises. Thus, the shape of the bright regions can be used to determine the variation of K_L and will be an important constraint on the thermoluminescence model. The eclipse light-curves are not sensitive to this effect. For example, if K_L varies after dawn like $K_L \sin \Omega t$ where Ω is the rotation frequency of Io and t the time since dawn, the brightness of the terminator is reduced to about $eF (K_L t_e)^{1/2}$ and

Fig. 1 Possible light curve of 'thermoluminescent' light from Io with parameters given in the text. If the thermoluminescent emission rate is proportional to the solar illumination, the bright dawn terminator effect is moved to the right and its intensity reduced.



the position of maximum intensity is pushed away from the geometrical terminator, to about $(\pi K_L t_0)^{1/2}$ radians, or 9° of longitude into the dayside of the terminator. Thus it is quite important that we have adequate resolution in λ_0 longitude to determine the position of the maximum brightness, and other details of the spatial dependence of this effect, so that the intensity of the effect may be properly interpreted. Particle flux levels measured at the same time will determine ϵ . The colour of the excess light should help to define the 'thermoluminescent' system. Comparison of the planned observations of the terminator 1 h apart (E. C. Stone, personal communication) will determine some spatial variations in ϵ and possibly other aspects of this process which might well occur due to uneven bombardment of λ_0 by the corotating energetic particle fluxes in the jovian magnetosphere.

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Millisecond
microwave spikes in a solar flare

THE shortest-lived structures in microwave solar flares known until now last ~ 1 s. Recently (11 April 1978) we observed a microwave outburst at 2.65 GHz (starting at 13.41 UT) showing a strong and extremely fast fluctuating burst component. Many spikes appeared to have half-power durations smaller than the 20-ms resolution of the instrument used. These are about two orders of magnitude shorter-lived than events reported from previous microwave observations. They are only rivalled by fine structure in some decimetric type IV bursts between 0.4 and 1.4 GHz (ref. 1).

The occurrence of microwave flares with millisecond fine structure is probably not very rare as we observed a second example on 28 April 1978 around 13.50 UT. Our resolution at

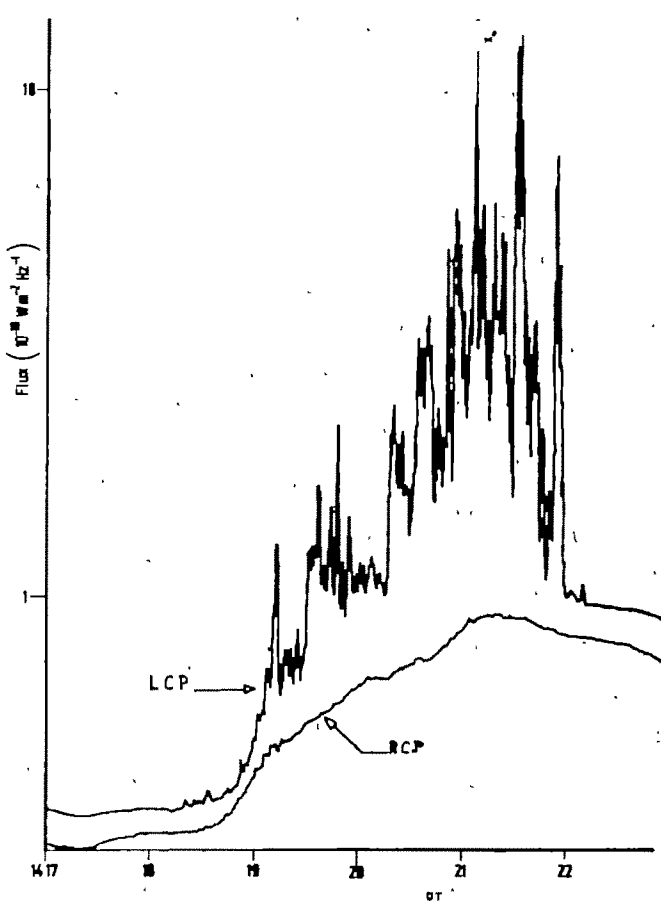
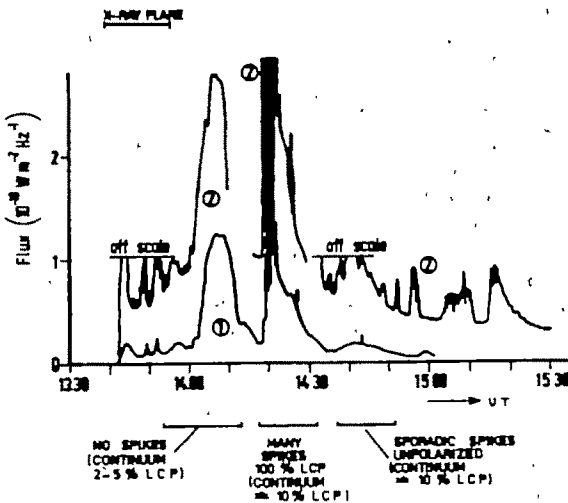


Fig. 2 Flux density of both left (LCP) and right (RCP) circularly polarised components of the most variable part of the event. The fine structure is not resolved. The indicated fragment (*) of about 2 s is expanded in Fig. 3. Note that this is not one of the most intense peaks but rather a typical one.

that time was limited to 100 ms, which turned out to be insufficient. The importance of this last flare in H_α was rather high, 4B, but that of the flare at 11 April had the more common rating 2B.

Our observations were made with a double sideband receiver sensitive to two bands of 12 MHz separated by 60 MHz. The receiver measures both left and right circularly polarised radiation (LCP and RCP), with an effective time constant of 10 ms. The outputs are recorded on stripchart recorders, allowing a maximum resolution of 100 ms, and, for full resolution, on magnetic tape with a sampling interval of 10 ms.

The microwave flare consisted of a large number of successive bursts (see Fig. 1). Some of these showed millisecond structure. The general behaviour on 2,650 MHz (Dwingeloo) corresponds very well with that on 2,800 MHz (Ottawa), but a sharp high frequency cut off is quite clear during the whole event. For the smooth component we found a fall of 3-5 dB over only 150 MHz. The fluctuating component is less easy to compare as the Ottawa time constant is much larger than the duration of the spikes. Comparison of this component after averaging over 0.5 s in both recordings yields a fall of 5-7 dB. The three major bursts (14.05, 14.20 and 14.40 UT) showed at 2,650 MHz a weakly L

Fig. 1 Reconstruction for H_α flare, 2B, N19W54, of the total power recording of 2,650 MHz flux observed at Dwingeloo (2) compared with that of 2,800 MHz flux observed at A.R.O. (1) (courtesy M. B. Bell at A.R.O., Ottawa). Data reconstructed from a logarithmic channel. The period of strong variability coincides for both frequencies. This period is shown expanded in Fig. 2.

polarised continuum, the first one without any spikes, the second one with a very large number of 100% L.C.P. spikes and the last one with sporadic, hardly (if at all) polarised, spikes (one might consider the weakly polarised continuum to be composed of spatially different L and R sources, but then these would have to show quite precisely the same temporal development; besides the occurrence of weakly polarised spikes sustains the concept of a weakly polarised source).

The spikes around 14.21 UT were analysed in detail. All were 100% L.C.P. Flux densities of up to $6 \times 10^{-19} \text{ W m}^{-2} \text{ Hz}^{-1}$ were observed for the spikes for nearly all durations (Fig. 3). The occurrence of these spikes starts along with the rise of the continuum, but ends rather abruptly (Fig. 2) long before the continuum died out.

The half-power durations of the spikes were almost all (95%) below 40 ms, while the abundance increased with decreasing duration (Fig. 4). This implies that many spikes were not resolved. The intervals between successive spikes were also rather short: 72% were below 50 ms and again their abundance was larger the shorter their value. There were no conspicuous periodicities but too many values were rather close to the sampling interval.

An important consequence is that the particle acceleration mechanism must be effective on the time scale of milliseconds, which is 2 or 3 orders of magnitude shorter than hitherto envisaged in most models^{2,3}. However, recently a pulsed

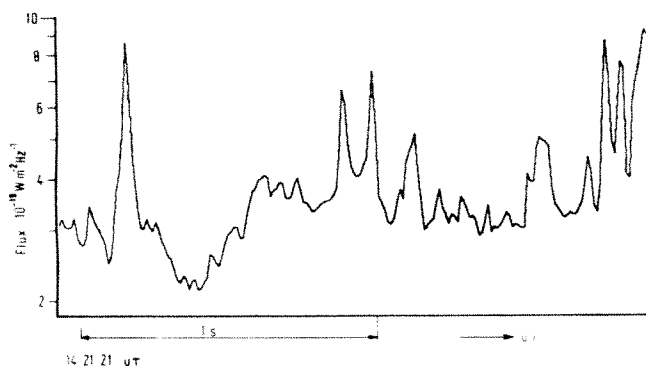


Fig. 3 Full resolution recording of a typical fragment of the fine structure. The intervals between measured flux values are 10 ms. Date, 11 April 1978.

acceleration process has been proposed⁴ where the time scales of the acceleration is the local collision time. Indeed the collision damping rate for plausible source conditions (kinetic temperature 10^7 K , particle density 10^{11} cm^{-3}) is a few milliseconds. Hence we assume the spike durations to be determined by the excitation periods. We can now estimate the source dimensions by assuming a spatial growth rate between the Alfvén speed and c . A plausible Alfvén speed would be $\approx 3,000 \text{ km s}^{-1}$ (field strength $\approx 300 \text{ G}$). A spike lasting 10 ms would then have a source width between 30 and 3,000 km. As an m.h.d. instability is the most likely generating process^{2,3}, dimensions of the order of tens of kilometres are a realistic estimate. Such values are considerably shorter than observed for microwave sources ($\approx 8,000 \text{ km}$)³ and H_α kernels (3,000–6,000 km), but have been hinted at in theoretical models^{2,3}.

The flux densities of the spikes, together with the source size estimates given above, lead to a lower limit of the brightness temperatures of $\sim 10^{15} \text{ K}$. Obviously a coherent radiation mechanism is necessary to account for such values. It is interesting to note that the completely polarised spikes can be explained as plasma radiation at the fundamental frequency, arising from induced scattering of Langmuir waves present in the pulsed acceleration model⁴, while the continuum burst can be explained as weakly polarised self-absorbed gyro-synch-

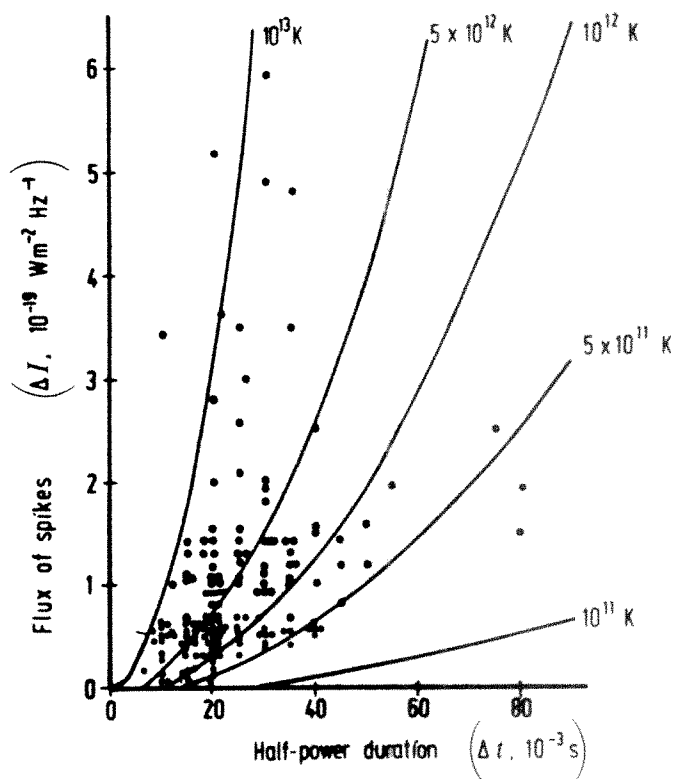


Fig. 4 Peak flux values of spikes plotted against their half-power durations. Curves of constant brightness temperature are drawn taking the Alfvén speed times the duration as typical dimension of the spike sources. Durations below 10 ms are cut off by the time constant of the receiver.

rotron radiation from the heated and accelerated particles all over the flare region. Observation of microwave spikes should provide direct evidence of the elementary flare processes.

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First mass analysis of stratospheric negative ions

ATMOSPHERIC negative ions have been identified previously by *in situ* mass spectrometric measurements only at altitudes above $\sim 70 \text{ km}$, which was the operational height limit of the rocket-borne instruments used^{1,2}. These measurements revealed the presence of atomic, molecular and cluster ions mostly having masses between 35 and 152 AMU. Here we report preliminary results of the first mass spectrometric measurements of stratospheric negative ions. Only observed mass numbers will be presented. A detailed analysis of the ion abundances, will be published elsewhere.

Three flights of a balloon-borne mass spectrometer probe were conducted during daytime over southwestern France (flight 1: 2 September 1977; flight 2: 26 September 1977; flight 3: 10 November 1977). Peak altitudes of 33.5, 35, and 37 km were reached. The instrument, developed and built at the Max-Planck-Institut für Kernphysik, has been previously described along with the circumstances of the flight measurements and the preliminary positive ion composition data obtained simultaneously³ and will not be reported here. Note, however, that the mass resolution setting of the instrument was lower for negative ions (~ 22) than for positive ions (~ 50) in favour of an increased transmission of the mass filter. Thereby a signal-to-noise ratio for negative ions comparable to that for positive ions should be achieved considering the higher dark count rate of the ion detection system in the negative ion measurement associated with the higher voltage applied to the channeltron.

Most negative ion data were obtained at the peak altitudes and only these will be reported here. As far as the major masses are concerned, the data obtained in the different flights agree with each other. The uncertainty of the mass identification up to ± 3 AMU is due to the limited definition of the flanks of the mass peaks. Laboratory and in-flight-calibrations of the mass scale were performed³. The width of a mass peak is typically about 15 AMU which implies that small mass peaks may easily become covered by large ones and thus may not be detectable.

The major ion masses (fractional abundance $> 1\%$) given in Table 1 cover the range from 125 to 295 AMU. When compared to the masses of the major positive ions observed in the same flights ranging from 55 to 140 ± 2 AMU (ref. 3), they are larger and are also greater than the negative ion masses observed above 70 km which were mentioned before.

Table 1 Mass numbers and tentative identifications of major negative ions observed at 33–37 km altitude

Mass	Tentative identification
125 ± 2	$\text{NO}_3^-\text{HNO}_3$
161 ± 2	R^-HNO_3 $\text{NO}_3^-\text{HNO}_3\text{HCl}$
188 ± 2	$\text{NO}_3^-(\text{HNO}_3)_2$
197 ± 3	R^-HR
224 ± 3	$\text{R}^-(\text{HNO}_3)_2$ $\text{NO}_3^-(\text{HNO}_3)_2\text{HCl}$
253 ± 3	$\text{NO}_3^-(\text{HNO}_3)_3$
260 ± 3	R^-HRHNO_3
289 ± 3	$\text{R}^-(\text{HNO}_3)_3$ $\text{NO}_3^-(\text{HNO}_3)_3\text{HCl}$
295 ± 3	$\text{R}^-(\text{HR})_2$

For identification of R see text.

An identification of the chemical nature of the observed negative ions is difficult at present mainly because of the limited mass accuracy, the lack of rate constants of relevant ion molecule reactions and the poor knowledge of stratospheric trace gases. Nevertheless, some general conclusions concerning the nature of the observed ions may be drawn.

The high mass numbers probably indicate cluster ions consisting of a core ion and attached ligand molecules. An upper mass limit of the least massive core ion is defined by the smallest major ion mass (125) observed. This is consistent with a core ion NO_3^- (62 AMU) being considered as the favoured core ion of stratospheric negative cluster ions because of the large electron affinity of NO_3^- (ref. 4). If the core ion is actually NO_3^- , mass 125 may be identified as $\text{NO}_3^-\text{HNO}_3$. The average mass of the building blocks of the cluster ions (core ion and ligand molecules) may be estimated by considering the following argument: the association order (number of ligand molecules attached to the core ion) of the negative cluster ions should be less than that of the $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ ions measured simultaneously. This has to be expected as the atmospheric concentration of the ligand molecules is probably lower than that of water and since the

bond energies of negative cluster ions inferred from laboratory measurements is generally lower than that of the $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ ions of the same association order (see ref. 5). A lower limit for the mass of the building blocks of about 74 ± 3 AMU may be estimated by considering a maximum association order of 3, the same as that of the observed $\text{H}_3\text{O}^+(\text{H}_2\text{O})_n$ ions and a maximum mass of the observed major negative ions of 295 ± 3 AMU. A large average mass of the ligand molecules is also inferred from the large mass difference of adjacent mass peaks. This rules out an identification of the observed negative ions as hydrates which were previously considered to dominate in the stratosphere⁶.

Our conclusion is supported by laboratory measurements of gas phase hydration equilibria of negative ions (see refs 7, 8; Fehsenfeld, personal communication; ref. 8) inferring that negative hydrates should contain only one or two water molecules at water vapour pressures and temperatures prevailing at 34–37 km altitude. Most likely, the ligand molecules are stratospheric trace gases which are much less abundant and which are stronger bound to negative ions than water.

The ligand molecules should have large gas phase acidities as laboratory studies indicate that the bond energy of a molecule to a negative ion increases with the gas phase acidity of the molecules⁹. Favoured candidates therefore seem to be, HI , HNO_3 , HBr , HCl , and H_2SO_4 . Cluster ions of the type $\text{NO}_3^-(\text{HNO}_3)_n$ which were previously proposed to exist in the stratosphere⁴ would be consistent with the observed masses 125 ± 2 ($\text{NO}_3^-\text{HNO}_3$), 188 ± 2 ($\text{NO}_3^-(\text{HNO}_3)_2$) and 252 ± 3 ($\text{NO}_3^-(\text{HNO}_3)_3$). The observed masses 161 ± 3 , 197 ± 3 , 224 ± 3 , 260 ± 3 , 289 ± 3 and 295 ± 3 seem to fit a pattern $\text{R}^-(\text{HR})_n(\text{HNO}_3)_n$ with HR having a mass of 98 ± 2 AMU and with $m+n \leq 3$ (Table 1). A tentative candidate for HR seems to be H_2SO_4 . Another possibility might be HClO_4 but this is unlikely to be sufficiently abundant in the upper stratosphere.

The identification of the observed ions deserves further investigation involving the determination of relative ion abundances, *in situ* measurements with higher mass resolution and laboratory studies of relevant ion reactions. Such measurements should also provide a base for an analytical application of stratospheric ion composition measurements in terms of a determination of the concentrations of certain neutral trace gases involved in stratospheric ion molecule reactions. If the observed ions would actually contain HNO_3 , H_2SO_4 and HCl , it should be possible to derive the concentrations of these gases from the ion composition. Thus, ion composition measurements may become a powerful analytical tool, which allows the detection of very small gas concentrations^{3,4,10}.

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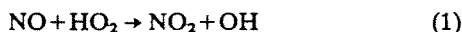
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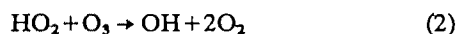
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Effect of the reaction $\text{HO}_2 + \text{O}_3 \rightarrow \text{OH} + 2\text{O}_2$ on stratospheric ozone

THE recent measurements¹⁻³ of large values (8.1 ± 1.5 and $8.2 \pm 2.7 \times 10^{-12} \text{ cm}^3 \text{ s}^{-1}$) of the rate coefficient for the reaction



have led to some important revisions in our understanding of stratospheric photochemistry⁴. In particular, the predicted ozone perturbations due to the injection of nitrogen oxides by supersonic transport aircraft have become net ozone gains instead of losses⁵, while the predicted ozone depletion due to ozone-active chlorine from chlorofluoromethanes has almost doubled⁴. An unfavourable result of the adoption of the larger value of the rate coefficient for reaction (1) has been the prediction of excessive amounts of stratospheric ozone (10–20% more than the measured concentrations) both in terms of the total column abundance and the concentration profile at altitudes below 40 km. Attempts to remedy this discrepancy between theory and observation by altering the simulated lower stratosphere transport and by increasing the assumed amount of ozone-active chlorine were not successful. It is the purpose of this letter, however, to show that another recent measurement by Zahniser and Howard⁶ of the rate coefficient, k_2 , for the reaction

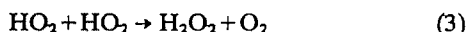


largely resolves these problems of excessive stratospheric ozone in models. Zahniser and Howard have reported the first direct measurement of the reaction between HO_2 and ozone (reaction 2). Their temperature-dependent rate coefficient is

$$k_2 = (1.4 \pm 0.4) \times 10^{-14} \exp [-(580 \pm 100)/T] \text{ cm}^3 \text{ s}^{-1}$$

which at stratospheric temperatures is 4 to 5 times larger than the value recommended in the report of the NASA Chlorofluoromethane (CFM) Workshop⁴, that is, $7.3 \times 10^{-14} \exp (-1,275/T) \text{ cm}^3 \text{ s}^{-1}$.

Before the work of Zahniser and Howard⁶ the only measurements of the rate coefficient for reaction (2) were the indirect competitive ones of Simonaitis and Heicklen⁷ and DeMore and Tschukow-Roux⁸, who measured the ratio $R = k_2/(k_3)^{1/2}$ where k_3 is the rate coefficient for the reaction



The measured ratios are presented in Table 1. To estimate the rate for reaction (2) from these ratios, one must have information about the value of k_3 . For example, the NASA CFM Workshop report⁴ recommended a temperature independent value for k_3 of $2.5 \times 10^{-12} \text{ cm}^3 \text{ s}^{-1}$ with an estimated uncertainty of a factor of 2 for reaction (3). This rate constant was based on the simultaneous consideration of the best available rate data for several related hydrogen radical reactions⁴. Cox⁹, however, has since reported a large negative activation energy for reaction (3); his measured rate constant is $(1.4 \pm 0.7) \times 10^{-14} \exp [(1,650 \pm 205)/T] \text{ cm}^3 \text{ s}^{-1}$. Based on the newest data collected for reaction (3) and the R values of Simonaitis and Heicklen⁷ and DeMore and Tschukow-Roux⁸, we can infer a much less marked temperature dependence for reaction (2) than was recommended in the CFM Workshop report⁴. Moreover, Hamilton *et al.*¹⁰ have found that the HO_2 radical involved in reaction (3) can readily cluster with water molecules—substantially accelerating the apparent rate of the reaction. Therefore the laboratory results obtained for R are expected to be complicated by HO_2 cluster reactions. Table 1 combines the kinetic data uncertainties for reaction (3) to estimate the 220 K (stratospheric) rate coefficients corresponding to each of the sets of available laboratory data. Note that the range of values of k_2 in Table 1 based on the measurements of Zahniser and Howard⁶

Table 1 Stratospheric $\text{HO}_2 + \text{O}_3$ reaction rate coefficient ($\text{cm}^3 \text{ s}^{-1}$)

Data source	$R = k_2/(k_3)^{1/2}$	k_2 (220 K) upper limit	k_2 (220 K) lower limit
Ref. 6	—	2.0×10^{-15}	4.5×10^{-16}
Ref. 4	—	4.4×10^{-16}	1.1×10^{-16}
Ref. 7	$1.2 \times 10^{-8} \exp (-1,000/T)$	$2.8 \times 10^{-16*}$	$1.4 \times 10^{-16*}$
		$1.3 \times 10^{-15}†$	$2.8 \times 10^{-16}†$
Ref. 8	$1.1 \times 10^{-7} \exp [-(1,550 \pm 250)/T]$	$6.7 \times 10^{-16*}$	$3.4 \times 10^{-17*}$
		$2.9 \times 10^{-15}†$	$6.9 \times 10^{-17}†$

* Based on the CFM Workshop⁴ recommended value for the rate coefficient for the reaction $\text{HO}_2 + \text{HO}_2$ of $2.5 \times 10^{-12} \text{ cm}^3 \text{ s}^{-1} \pm$ factor of 2.

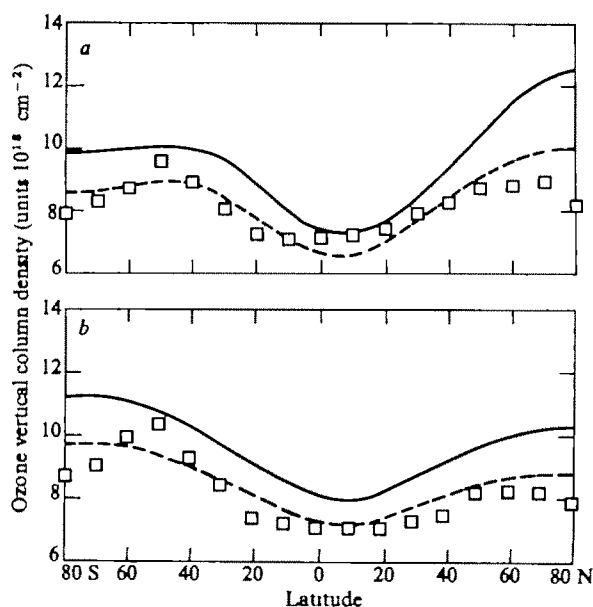
† Based on Cox's⁹ rate coefficient for the reaction $\text{HO}_2 + \text{HO}_2$ of $(1.4 \pm 0.7) \times 10^{-14} \exp [(1,650 \pm 205)/T] \text{ cm}^3 \text{ s}^{-1}$.

roughly coincides with the range of values deduced from the ratio measurements when Cox's effective rate for reaction (3) is adopted. In this sense, the previous measurements support the new, larger value for the rate coefficient k_2 .

We use a two-dimensional model of stratospheric trace constituents for our calculations^{11,12}. The model lies in a meridional plane extending from 80° N to 80° S latitude and from the Earth's surface to 60 km altitude. It computes the concentrations of 35 constituents and simulates meridional and vertical transport by seasonally variable large scale bulk motion and eddies. Except for reaction (2), the chemical rate coefficients employed are those recommended in the NASA CFM Workshop report⁴.

Figure 1 compares the measured latitude-dependent total ozone column densities in summer (a) and in autumn (b) in the Northern Hemisphere¹³ with the corresponding computed densities for both the old⁴ and new⁶ rate coefficients for reaction (2). Figure 2 shows two of the corresponding vertical concentration profiles of ozone at 0° and 45° N latitude in autumn for these two rate coefficient values of reaction (2). Note that the agreement between theory and measurement has improved more at high latitudes than at low latitudes. This latitude

Fig. 1 Total vertical column densities for a, summer and b, autumn in the Northern Hemisphere. The solid lines correspond to the CFM Workshop⁴ recommended value of the rate coefficient for the reaction of HO_2 with O_3 . The broken lines correspond to the rate coefficient reported by Zahniser and Howard⁶. The squares represent mean seasonal measured values collated by Wilcox *et al.*¹³.



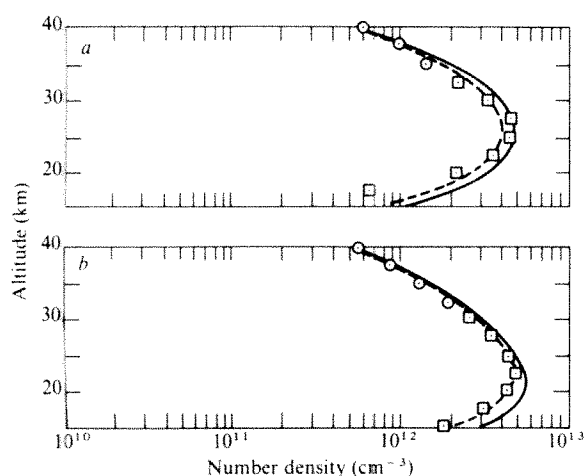


Fig. 2 Computed and measured ozone concentration profiles at latitudes of *a*, 0° and *b*, 40° N for autumn in the Northern Hemisphere. The squares represent the mean seasonal measured values collated by Wilcox *et al.*¹³. The Goddard data (circles) (I. Eberstein, personal communication) were obtained from satellite measurements of backscattered solar ultraviolet radiation¹⁴. The solid lines correspond to the CFM Workshop⁴ recommended value of the rate coefficient of the reaction of HO₂ with O₃ and the broken lines to the value reported by Zahniser and Howard⁶.

difference occurs because reaction (2) represents a larger fraction of the total ozone loss rate at the higher latitudes.

Reaction (2) represents the dominant catalytic loss process for ozone below 30 km. The dominance of this reaction over NO_x catalytic reactions results mainly from the new reaction rate coefficient for reaction (1). Poppoff *et al.*⁵ discuss the details of the coupled HO_x-NO_x photochemical cycles in the stratosphere for updated chemistry. They also give a sensitivity analysis (of total ozone to the injection of NO_x by supersonic transport aircraft) which is relevant to a study of the effects caused by changes in the HO₂ + O₃ reaction rate constant. Basically, since HO_x chemistry exerts strong control over the ozone abundance in the lower stratosphere, increasing the strength of the HO_x catalytic cycle [by increasing the rate constant for reaction (2)] tends to decrease the stratospheric ozone content.

Thus, we have found that the problem presented by the model predictions of excessive ozone abundances, which occurs when the recently measured value of the rate coefficient for reaction (1) is used, diminished significantly when the newly measured value of the rate coefficient for reaction (2) reported by Zahniser and Howard⁶ was used.

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Effect of pressure on the Raman spectrum of ice

THE decrease in the volume of ice on compression or cooling is thought to be due to a decrease in the O-O distances, because the O-O-O and O-H...O angles do not change. The decrease in the IR or Raman frequency of the in-phase O-H stretching vibrations, ν_1 , of ice is thus related to the consequent shortening of the hydrogen bond. It follows that ν_1 should remain constant at a constant volume, if anharmonic effects and the bending of O-H...O angles are insignificant. We report here the effect of pressure on the Raman spectral region of O-H stretching in hexagonal ice and discuss it in terms of explicit temperature and volume effects.

A cylindrical steel vessel with three 1-cm thick sapphire windows was used as the high pressure optical cell. The 5-mm diameter, 20-mm long sample of polycrystalline ice was mounted on the inside of one sapphire window with the cylindrical axis along the direction of the laser beam of power level 700-800 mW of the 4,880-Å excitation line. The optical cell was filled with Dow-Corning silicone oil, which acted as the pressure-transmitting fluid. The scattered light was collected at 90° to the laser beam and its intensity was measured with a calibrated Jarrel-Ash model 25-400 laser Raman spectrometer using a spectral slit width of 7-8 cm⁻¹.

Typical Raman spectra in the O-H stretching region (2,800-3,600 cm⁻¹) are given in Fig. 1. The features of the spectrum at high temperatures are relatively broad, because of the increase in the distribution of energy states, but they are essentially similar to those in the spectra measured below 100 K in our own study, or in the literature^{1,2}. In Fig. 1, the spectral features of ice remain unaffected by the presence of silicone oil, but two additional peaks appear at about 2,904 and 2,966 cm⁻¹ due to the C-H stretching mode in silicone. Application of pressure to ice at 258 ± 0.3 K decreases the frequency of the O-H stretching peak from 3,145 cm⁻¹ at 1 bar to 3,127 cm⁻¹ at 1.25 kbar.

A plot of ν_1 against pressure P at 255.2 ± 0.3 K is shown in Fig. 2. The $(\partial\nu_1/\partial P)_T = -13.7$ cm⁻¹ kbar⁻¹, a value which shows little systematic dependence on temperature in the region 269 > T > 253 K. A plot of ν_1 against temperature at 1 bar is also shown in Fig. 2. The $(\partial\nu_1/\partial T)_P = 0.63$ cm⁻¹ K⁻¹.

The effect of temperature on ν_1 at constant pressure may be divided into the constant-volume temperature effect, $(\partial\nu_1/\partial T)_V$, and the constant-temperature volume effect, $(\partial\nu_1/\partial V)_T$ by the equation

$$(\partial\nu_1/\partial T)_P = (\partial\nu_1/\partial T)_V + (\partial\nu_1/\partial V)_T(\partial V/\partial T) \quad (1)$$

$$(\partial\nu_1/\partial V)_T(\partial V/\partial T) = -(\alpha/\beta)(\partial\nu_1/\partial P)_T \quad (2)$$

where α is the volume expansivity and β the compressibility. At 255 K, $\alpha = 155$ MK⁻¹ (ref. 3) and $\beta = 12 \pm 1$ Mbar⁻¹ (ref. 4). From Fig. 2, $(\partial\nu_1/\partial P)_T = -13.7$ cm⁻¹ kbar⁻¹, and $(\partial\nu_1/\partial T)_P = 0.63$ cm⁻¹ K⁻¹. Substituting in equations 1 and 2, one obtains $(\partial\nu_1/\partial T)_V = 0.45$ cm⁻¹ K⁻¹. Thus the change in the O-H stretching frequency of ice with temperature near 255 K is about 30% due to the change in volume with temperature and about 70% due to the change in temperature alone. Thus the Grüneisen constant for the O-H stretching vibrations, $\gamma = -(\partial \ln \nu_1 / \partial \ln V)$, calculated from the above values is -0.36 at 255 K.

From the compressibility of ice⁴, the change in O-O distance with pressure, $(\partial R_{O-O}/\partial P)_T = -9$ Å Mbar⁻¹ at 255 K, where R_{O-O} is the O-O distance. From the R_{O-O} data⁵ adjusted to the

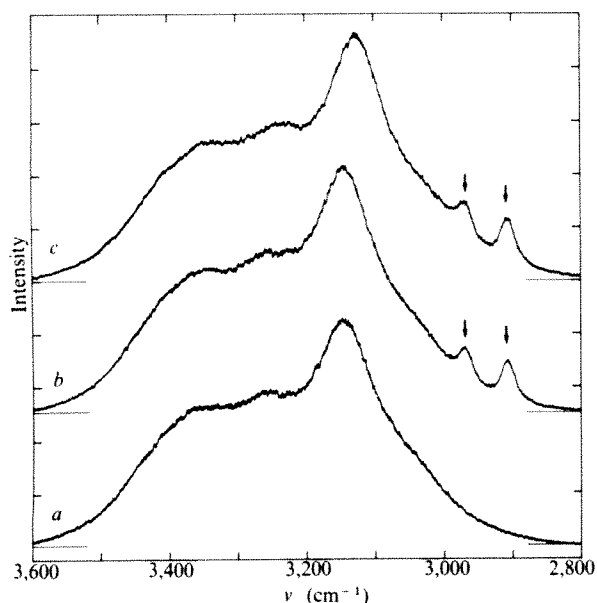
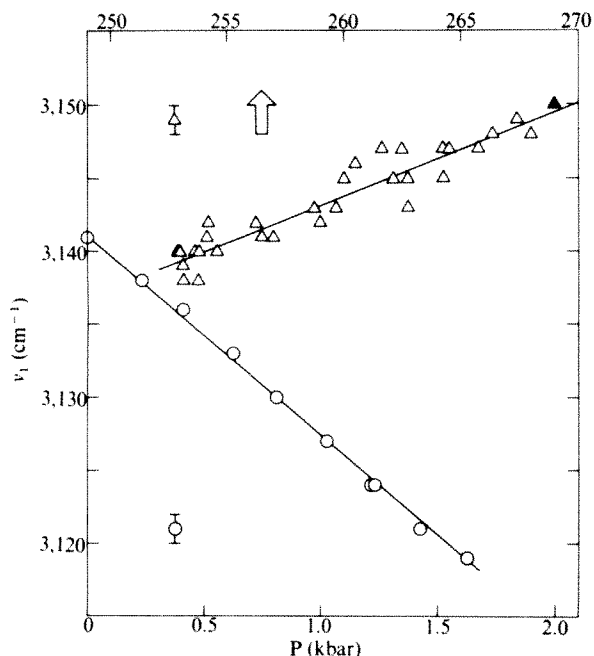


Fig. 1 Raman spectrum of hexagonal ice at 258 ± 0.2 K in the O-H stretching region. *a*, Ice held in air; *b*, immersed in silicone oil at 1 bar; and *c*, immersed in silicone oil at 1.25 kbar. Arrows point to the frequencies of the C-H stretching in silicone. Note also the slight increase in the C-H stretching frequencies with pressure.

density calculated from crystallographically determined expansivity³ of ice, the change in R_{O-O} with temperature, $(\partial R_{O-O}/\partial T)_P = 116 \text{ Å MK}^{-1}$ at 255 K and 1 bar. Combining them with the values of the slope of the plots in Fig. 2, one obtains $(\partial \nu_1/\partial R_{O-O})_P = 5,430 \text{ cm}^{-1} \text{ Å}^{-1}$ and $(\partial \nu_1/\partial R_{O-O})_T = 1,520 \text{ cm}^{-1} \text{ Å}^{-1}$. So, for the same amount of decrease in O-O distance, isobaric cooling decreases ν_1 3.5 times more than does isothermal compression near 255 K.

The increase in ν_1 with temperature at a constant volume (or constant R_{O-O}) is of principal interest. The relatively high

Fig. 2 The frequency of the in-phase O-H stretching vibration, of hexagonal ice plotted against temperature at 1 bar at the top and against pressure at 255 ± 0.3 K at the bottom. Filled triangle is from an isotropic spectrum from ref. 12.



positive value of $(\partial \nu_1/\partial T)_V$ indicates either or all of the following three: (1) the lengthening of the hydrogen bond with increasing temperature; (2) an unusual effect due to the anharmonicity of the potential energy of O-H stretching; (3) the weakening of the intermolecular coupling of O-H stretching motions, when the O-O distance remains constant. The lengthening of the hydrogen bond can occur either by the bending of O-H...O angles or (but less likely) by a shortening of O-H distance if the O-H...O bond were to remain linear. The force constant for the O-H...O bending in ice is small⁶ and the IR frequencies of librational modes indicate that the energy required to bend the O-H...O angle becomes smaller as the temperature increases. If lengthening of the hydrogen bond occurs, the energy required to break the hydrogen bond would decrease and the activation energy of dielectric relaxation at a constant volume should therefore decrease with increasing temperature. The following consideration shows that it is so. The activation energy at constant volume, E_v^* , and constant pressure, E_p^* are related by

$$E_p^* = E_v^* + (\alpha/\beta)T\Delta V^* \quad (3)$$

where ΔV^* is the volume of activation. Differentiating equation (3) with respect to temperature:

$$(\partial E_p^*/\partial T)_P = (\partial E_v^*/\partial T)_V + (\alpha/\beta)\Delta V^* + (T\Delta V^*/\beta)(\partial \alpha/\partial T)$$

For ice near 255 K, $\Delta V^* = 3 \text{ ml mol}^{-1}$ (ref. 7), $(\partial \alpha/\partial T)_P = 7.39 \times 10^{-7} \text{ K}^{-2}$ (ref. 8), and $(\partial E_p^*/\partial T)_P$ is zero⁹. Therefore, $(\partial E_v^*/\partial T)_V = -9 \text{ J mol}^{-1} \text{ K}^{-1}$.

The simplest consideration of anharmonicity leads one to a negative value of $(\partial \nu_1/\partial T)_V$, for anharmonicity generally reduces the force constant and consequently the frequency of a vibrational mode, when a system is excited to higher energy levels by raising the temperature at a constant volume. It is conceivable that terms higher than the cubic predominate in the potential energy of O-H stretching and thus anharmonicity may play a greater role in determining the temperature dependence of ν_1 . The intermolecular coupling of O-H vibrations at a constant volume is affected by both the lengthening of the hydrogen bond and the anharmonicity of the potential energy. Its magnitude is difficult to assess without the knowledge of the other two effects.

The above results are significant because it is widely believed that the vibrational frequencies depend largely on the volume and because the results may be helpful in a detailed analysis of the vibrational spectrum of both the crystalline^{10,11} and vitreous ices² from the point of view of solid state spectroscopy. They are of further value, because the corresponding measurement of the IR spectra of hexagonal ice is difficult.

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Collection of atomic mercury by electrostatic precipitators

ELECTROSTATIC precipitators are widely used to remove dust particles from gases and the mechanism of this is well understood. One major application is in the cleaning of a sulphur dioxide produced in smelting of metalliferous sulphide ores before its conversion to sulphuric acid and the precipitated material is often found to contain mercury. We have shown that the concentration of mercury is below the saturation level and therefore present as atoms so the accepted mechanism for solid particle collection is not involved in mercury removal. We have investigated the phenomenon in the laboratory using air as the carrier gas at room temperature, and by large scale tests carried out in an 800 tonne d^{-1} sulphuric acid plant treating the gases formed by roasting zinc sulphide concentrate. In both types of test a discharge wire and earthed collector tube precipitator configuration was used. In the plant tests the roaster gas contained 9% sulphur dioxide and was saturated with water. In both the laboratory and plant tests the gas streams were unsaturated with respect to elemental mercury vapour.

Flameless atomic absorption spectroscopy was used to measure the difference in the mercury concentration of gas entering and leaving a precipitator. This technique is specific for the detection of discrete gaseous atoms, which suggests that gaseous atomic mercury, and not particles containing mercury, is removed by electrostatic precipitators.

In our tests, mercury was only collected during or after generation of a corona discharge (Fig. 1). When nitrogen was used as a carrier gas, mercury was not collected because nitrogen will not sustain a corona discharge¹.

We postulate that in the corona discharge, high energy ions bombard the electrode surfaces and disrupt a passivating layer, such as an oxide, hence producing a more reactive surface. When mercury atoms diffuse to the electrode surface they are adsorbed on the active sites.

Mercury was collected by both electrodes, irrespective of discharge wire polarity, and by a variety of electrode materials including lead, copper, tin, aluminium and stainless steel.

Following voltage application, mercury collection efficiency increased gradually to a steady-state value which was independent of the electrode material. The time taken to achieve the steady-state condition with respect to the mercury collection varied with electrode material (Fig. 1), reflecting the time required to clean electrode surfaces of different chemical composition. The extent of cleaning, and hence the steady-state mercury collection efficiency, depended on the voltage. Mercury

Fig. 1 Time dependence of elemental mercury collection in an electrostatic precipitator. *a*, Voltage on 880 kV m^{-1} ; *b*, voltage off, air velocity, 0.084 m s^{-1} . $\times$, Copper tube; $\circ$, aluminium tube both 35 mm diameter.

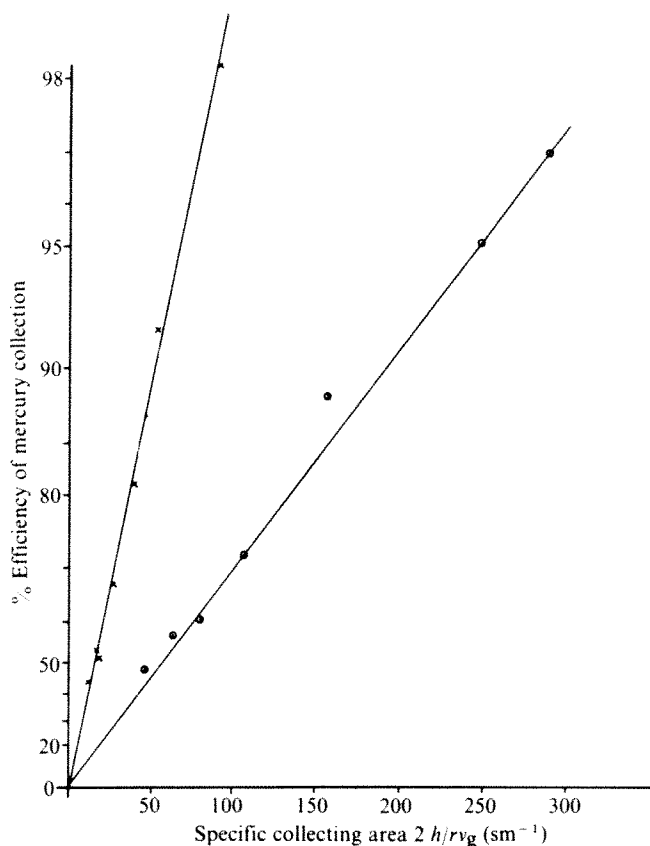
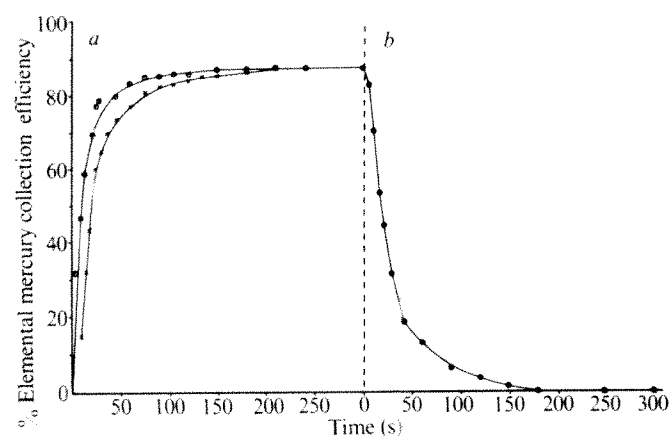


Fig. 2 Variation of mercury collection efficiency with precipitator specific collecting area at constant electrical field strength. $\times$, Tube radius 8 mm, electrical field strength 800 kV m^{-1} ; $\circ$, tube radius 23 mm, electrical field strength 520 kV m^{-1} .

collection continued after the voltage had been switched off (Fig. 1) the efficiency of collection decreasing continuously to zero as the electrode surfaces passivated. The rate at which the efficiency of mercury collection decreased was substantially slower than the decay of the precipitator voltage. If the collection of mercury ions was involved, mercury collection efficiency would have decreased discontinuously when the voltage was switched off, because ions would only be formed when the voltage was applied.

For a precipitator operating with a negative discharge wire and an earthed collector tube, the efficiency of atomic mercury collection can be described by equation (1), which is derived from the Deutsch equation²

$$E = 100[1 - \exp(-k2h/rv_g)] \quad (1)$$

where E is the percentage efficiency of mercury collection, r and h are the collector electrode tube radius and length, respectively, v_g is the axial gas velocity, and $(2h/rv_g)$ is the specific collecting area of the precipitator (electrode surface area/gas flow rate), and k is a complex function incorporating the rate of transport of mercury atoms to the electrode surfaces and their probability of collection. k varies with tube radius and voltage according to equation (2)

$$k = AV/2r \quad (2)$$

where A is a constant for a given tube radius and V is the applied voltage.

Equation (1) was verified experimentally (Fig. 2) over a wide range of conditions ($r=8.0$ – 45 mm for laboratory and $r=75$ mm for plant tests). A maximum mercury collection efficiency in excess of 99% was achieved. The efficiency of

mercury collection was independent of the mercury concentration in gas entering the precipitator for concentrations in the range 0.1–8 mg m⁻³.

At very high discharge voltages equation (1) may over-estimate mercury collection efficiency because the gaseous ions bombarding the electrode surfaces can become sufficiently energetic to desorb collected mercury. In the absence of a corona discharge, no mercury was released when mercury-free air was passed through a precipitator which had previously been used to collect mercury, proving that mercury was bound to the electrode surfaces. However, when a discharge voltage greater than the threshold value for which equation (1) is valid was applied, mercury was desorbed into the mercury-free air. The threshold voltage for mercury desorption was independent of electrode material.

For precipitators operating at a constant voltage below the threshold voltage for mercury desorption, A and hence k (equations (1) and (2)) increases with decreasing tube radius for $r < 35$ mm, but seems to be independent of tube radius when $r > 35$ mm. Because k increases as r decreases below 35 mm the electrode area required for a given application can be decreased by designing a precipitator with a very small electrode separation.

We suggest that electrostatic precipitators with smaller than normal electrode separations would be capable of removing mercury and perhaps other volatile environmentally undesirable elements, such as selenium and tellurium, which are present as vapour in the gas streams produced during the processing of ores and the burning of certain types of coal.

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Molecular morphology of polyethylene determined by NMR

SOME of the earliest applications of nuclear magnetic resonance (NMR) concerned molecular structural determinations in the solid state via proton NMR second moments, a sensitive function of the number, types and relative positions of the constituent nuclei. This classical technique has been extended to polymeric solids, but it has not previously been exploited to address the problem of molecular morphology in polymer crystals. We describe here a framework for the interpretation of such measurements in cocrystals of polyethylene (PEH) and perdeuteropolyethylene (PED) and present preliminary experimental data. The potential of this technique for distinguishing between adjacent re-entry and random re-entry molecular morphologies on a very local scale complements the ability of small angle neutron scattering (SANS) to ascertain the larger scale configuration of the polymer chain.

The bulk morphology in semicrystalline polymers is well understood. It can be described superficially as a dispersion of crystalline lamellae (with dimensions of the order of 10⁻⁶ m on the edges and 10⁻⁸ m in thickness) in an amorphous matrix. By contrast, only a limited number of features of the molecular morphology within the lamellae have been unequivocally defined^{1,2}, and these are all functions inherent in the primary

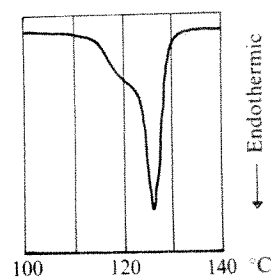


Fig. 1 The melt endotherm for an etched, solution-crystallised polyethylene co-crystal with $x^0 = 0.53$; heating rate 5°C per min; DuPont 990 thermal analyser. The PED aggregates in the co-crystal. The low temperature shoulder, approximately 15% to 25% of the total endotherm, is attributed to the PED phase resulting in a range for the mole fraction of PEH in the co-crystal, $x = 0.64$ to 0.72 . (Note, our observed melting point for quenched, unetched PEH is 136°C.)

structure of the polymer: (1) the extended, low energy chain conformation; (2) the lateral juxtaposition of the chains; and (3) the orientation of the rectilinear chain axis normal to the lamella surface.

The latter feature has prompted controversial hypotheses to explicate the mode of re-entry of a polymer chain into the lamella surface, a 001 lattice plane. The adjacent re-entry (AR) model posits a series of regular folds (180° turns) that dictate re-entry of the chain into the lamella at an adjacent, nearest-neighbour site in the 001 plane³. The random re-entry (RR) or 'switchboard' model imposes no such external constraints on the trajectory of the chain. The RR model stipulates that, on emerging from the lamella, the chain either (1) re-enters the same lamella at a random site, which could be far removed from the point of emergence (subject to the stereochemical and linear dimensional constraints of the polymer chains) or, (2) permeates the interlamellae amorphous matrix and enters a neighbouring lamella^{4,5}.

Recent SANS investigations of co-crystallised mixtures of PED and PEH have rekindled this controversy⁶ and have been used as supporting evidence for both the AR^{7,8} and the RR^{9,10} models, depending on the method of sample preparation. By way of circumventing this interpretive dilemma we pursue a different experimental approach to the problem—NMR, a sensitive technique for probing the local environment of a molecule. Proton NMR second moment measurements¹¹, typically used to investigate the bulk morphology¹² and relaxation processes¹³ in semicrystalline polymers, provide an entree to the problem.

For protons in a polycrystalline sample, the second moment is¹⁴

$$M_2 = \frac{3}{4} \gamma^2 \hbar^2 I(I+1) \frac{1}{N} \sum_{j,k} (r_{jk})^{-6} \equiv M_2^a + M_2^i \quad (1)$$

where γ is the proton gyromagnetic ratio, its spin $I = 1/2$, N is the number of nuclei in the sum and r_{jk} is the distance between nuclei j and k . In equation (1) we decompose M_2 into its intramolecular part M_2^a and its intermolecular part M_2^i . The former can be calculated from the molecular structure and conformation, the latter from the crystal structure. (In the polymer crystal, we will consider the analogous quantities, the intra- and interstem contributions to M_2 , where the term 'stem' refers to a single traverse of the chain through the lamella.) Non-resonant nuclei I' can also contribute to M_2 but with an additional reduction factor (relative to protons)¹⁵:

$$M_2' = \frac{4}{9} \frac{I'(I'+1) \gamma'^2}{I(I+1) \gamma^2} M_2 \quad (2)$$

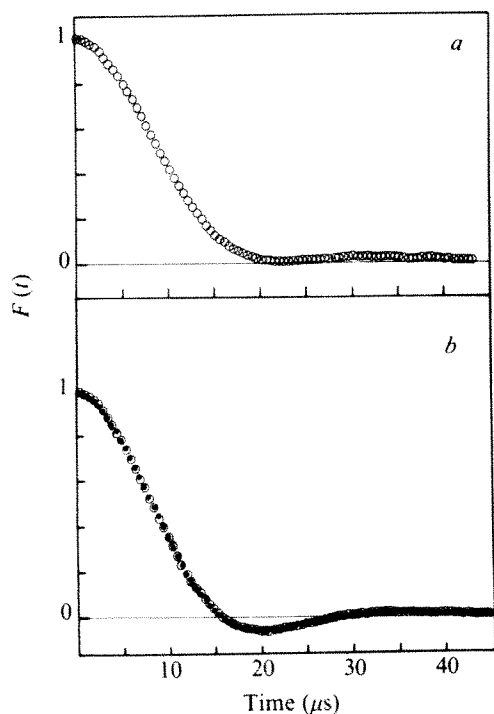
where I' and γ' are characteristic of the non-resonant nucleus.

For deuterium ($I' = 1$), this reduction amounts to $\cong 0.02$ of that anticipated from protons at comparable r_{jk} .

Samples of co-crystallised PEH and PED (molecular weights 2.0×10^5 and 2.8×10^5 , respectively) were prepared in two ways: in Method I, quenched polyethylene, 1% solutions of mixtures of PEH and PED ($x = 1$ to 0.28; x = mole fraction of PEH) refluxed in orthodichlorobenzene for 30 min under N_2 were quenched (precipitated) in an excess of methanol, washed with methanol and dried under vacuum at 65 °C (48 h); in Method II, solution-crystallised polyethylene, a 0.1% solution of PEH and PED ($x = 0.53$) in refluxing orthodichlorobenzene was rapidly cooled (~ 2 min) to 90 °C and allowed to stand (48 h). The resulting crystals were collected at 90 °C, extracted with acetone in a Soxhlet (24 h) and dried as in Method I. In order to exclude contributions to M_2 originating from the amorphous fraction of the polymer (see Fig. 2) all samples were subjected to a fuming nitric acid etching procedure (80 °C for 96 h); the highly crystalline debris was washed in H_2O , extracted with acetone (24 h) and dried under vacuum at 65 °C (24 h).

For all co-crystals calibrated NMR intensity measurements show that $\bar{x}$, the mean mole fraction of PEH in the co-crystal, is identical to that initially present in the solution blend, x_0 , that is, PEH (PED) is not partitioned preferentially into the amorphous phase and lost during etching. Aggregation of PEH (PED) into distinctly separate phases can be checked by examining the melting endotherms of the co-crystals⁹. Single endotherms are observed for quenched, etched co-crystals. A shoulder on the low temperature side of the endotherm is indicative of PED aggregation in the co-crystal. In the case of solution-crystallised mixtures of PEH and PED, the aggregation of the PED is pronounced (Fig. 1). In the resulting co-crystal, the local mole fraction of PED, x , is obviously not the same as x_0 . We have graphically decomposed this endotherm into two components, the melting of a PEH/PED co-crystal at 126 °C and the melting of aggregated PED at 122 °C. We estimate that the PED aggregate accounts for 15% to 25% of the entire endotherm

Fig. 2 The proton FID signal in polyethylene following a $90^\circ_0 - \tau - 90^\circ_{90}$ pulse sequence. The signals were obtained with a Bruker pulse spectrometer at 30 MHz; 90° pulse length = 1.5 μ s; $\tau = 10$ μ s; $T = 20$ °C. Typically 256 FID signals were averaged using a Biomation 802 digitiser and a Nicolet 1072 signal averager. $\circ$, Experimental FID. *a*, Quenched PEH. *b*, Quenched and etched PEH: $\bullet$, computed FID



and, assuming the same ΔH for PEH and PED, x ranges from 0.64 to 0.72 for this co-crystal. From the melting points of these co-crystals we estimate lamellae thicknesses of 150 Å and 180 Å for solution quenched and solution-crystallised samples, respectively¹⁶.

The NMR absorption line shape and the free induction decay (FID), $F(t)$, are related by a Fourier transform. Even moments M_n of the resonance line shape are related to the coefficients of an even power series expansion of $F(t)$ as¹⁵

$$F(t) = \sum_{n, \text{even}} \frac{M_n t^n}{n!} \quad (3)$$

A fit of the observed FID to this power series was used to compute the second moments. Figure 2 contrasts the FIDs of unetched and etched PEH and illustrates a computed fit to equation (3). The value for M_2 obtained from quenched PEH at 20 °C is $26.3 \pm 0.5 G^2$, and lowering the temperature to -150° produced no observable changes in M_2 . As noted previously, when the amorphous fraction is etched from PEH, the rigid lattice limit obtains at 20 °C (refs 17, 18). The observed M_2 is, however, larger than that previously reported for etched PEH after correcting for thermal expansion of crystal lattice ($24.3 \pm 0.5 G^2$).¹⁷ While the reproducibility of M_2 is excellent, the absolute magnitude computed from the FID is limited by the accuracy of determining the position of the dipolar echo ($t = 0$) following the $90^\circ_0 - \tau - 90^\circ_{90}$ pulse sequence. Once a procedure for determining $t = 0$ is established, relative values of the second moment, $R = M_2/26.3$, can be confidently compared.

Isotopic dilution experiments can be used to separate R into the intra- and interstem contribution to M_2 , R^a and R' , respectively. The interstem contribution is scaled by the conditional probability $P(H|H)$ that a given nearest-neighbour (site) of a protonated species is also protonated. In order to extract the details of the molecular morphology in the lamellae we calculate S , the interstem contribution to M_2 per site in the crystal relative to that for pure PEH, for the following models of chain re-entry: (1) AR, adjacent reentry with no restrictions on the direction of chain propagation in the 001 plane of the lamella; (2) AR-110, adjacent re-entry with the chain restricted to enter the lattice along the 110 planes; (3), AR-100, adjacent re-entry restricted to the 100 planes; (4) RR, random re-entry of the chain.

In the 001 plane of the crystal each lattice site has six adjacent sites, two along the 100 plane and two each along the 110 and $\bar{1}\bar{1}0$ planes. The absolute computed interactions along the crystal planes, $^{100}M'_2$ and $^{110}M'_2$ depend on the lattice cell constants. However, over a range of cell dimensions the relative interactions $^{ijk}R' = ^{ijk}M'_2/M'_2$ remain invariant: $^{100}R' = 0.13$ and $^{110}R' = ^{1\bar{1}0}R' = 0.186$.

For the AR model, around each PEH site at least two adjacent sites must also contain PEH chains ($P(H|H) = 1$, ignoring chain ends). We assume that chains with just two self-adjacent sites occur with a probability of 1/3. For multiple self-adjacent re-entries (3, 4, 5, or 6) about a given site we assign a probability of 1/6. The relative contribution S is obtained from the probability weighted sum of $^{ijk}R'$ arising from the different possible configurations about a given site. For the above parameters, one obtains

$$S(\text{AR}) = \frac{22}{36} + \frac{14}{35}x \quad (4)$$

In the AR-110 model, $^{110}P(H|H) = 1$ and $^{100}P(H|H) = ^{1\bar{1}0}P(H|H) = x$. Thus

$$S(\text{AR-110}) = 2^{110}P(H|H)^{110}R' + 2[^{1\bar{1}0}P(H|H)^{1\bar{1}0}R' + ^{100}P(H|H)^{100}R'] \quad (5)$$

$$= 0.37 + 0.63x$$

Similarly, $S(\text{AR-100})$ is given by

$$S(\text{AR-100}) = 0.26 + 0.74x \quad (6)$$

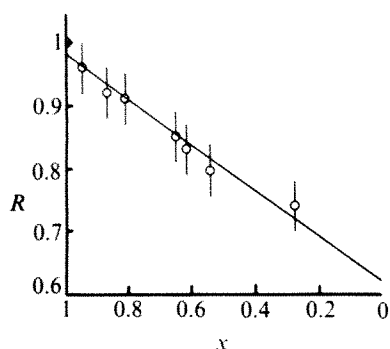


Fig. 3 The relative second moment R plotted against x for solution-quenched and etched co-crystals of PEH and PED; $x = \bar{x} = x_0$ for all samples. The solid line is a linear regression fit to the data with a correlation coefficient of 0.86; $R(x=0) = 0.62 \pm 0.04$; $R(x=1) = 0.98 \pm 0.04$.

Finally, in the RR model, where $P(H|H) = x$ for all sites we obtain

$$S(\text{RR}) = x \quad (7)$$

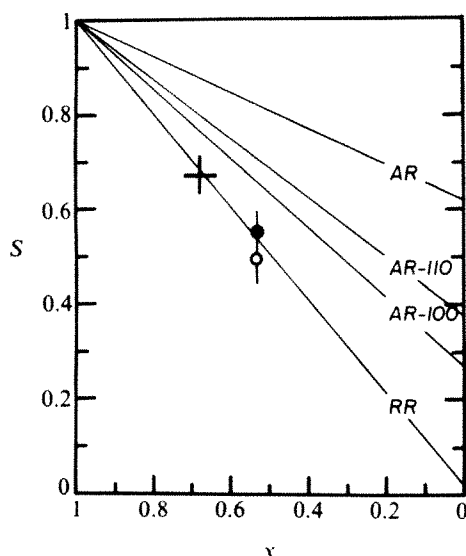
In each of these models the contribution that PED chains in adjacent sites makes to M_2' can be obtained with equations (4)–(7) by replacing x with $(1-x)$ and multiplying the result by 0.02.

To determine which model corresponds to the experimental data, we consider the behaviour of the relative second moment with the isotopic content of the co-crystals. R should be a linear function of x for all the models if the co-crystals are homogeneous. The intercept consists of the intrastem contribution plus the residual interstem contributions characteristic of the molecular morphology. Our procedure is to compute R^a from the intercept of R versus x for the quenched PEH/PED co-crystals (Fig. 3) and the expressions for S of equations (4)–(7) according to

$$R^a = [(R - S)/(1 - S)]_{x=0} \quad (8)$$

The values of R^a computed in this fashion are 0.02, 0.40, 0.47, and 0.62 ± 0.04 respectively, for the AR, AR-110, AR-100 and

Fig. 4 The relative intermolecular contribution to M_2 , S , plotted versus x , the mole fraction of PEH in co-crystals of PEH + PED. $\circ$, Quenched, etched co-crystal; $x = x^0 = 0.53$. $\bullet$, Quenched, annealed (123 °C; 48 h), etched co-crystal; $x = x^0 = 0.53$. $+$, Solution-crystallised, etched co-crystal; x ranges from 0.64 to 0.72 (see Fig. 1).



RR models of chain re-entry. These values of R^a can be compared with the values for R^a calculated for plausible structural and lattice parameters. It should be noted that calculated values for R^a are extremely sensitive to the choice of the bond angles and bond lengths used to compute M_2' (ref. 13). The calculated values of R^a range from 0.67 to 0.73. Thus, from the magnitude of the intercept, there is strong evidence for a random molecular dispersion of PEH in PED characterised by the RR molecular morphology in the quenched co-crystals. The observed $R^a = 0.62 \pm 0.04$ is lower than anticipated and probably reflects, as suggested by Olf and Peterlin,¹⁷ a decrease in M_2' caused by the presence of $-\text{COOH}$ and $-\text{NO}_2$ groups near the chain ends at the etched lamella surface. On the other hand, heterogeneity due to phase separation in the co-crystals would, for a given x^0 , yield larger experimental values for R and result in a correspondingly larger apparent R^a .

Now we consider the relative interstem contributions to M_2 . In Fig. 4 the calculated values of S (equations (4)–(7)) are shown plotted against x for the four models of chain re-entry. Also shown are three experimental values for relative interstem second moments derived from $S = (R - R^a)/(1 - R^a)$ with $R^a = 0.64 \pm 0.04$. The experimental values correspond to PEH/PED co-crystals ($\bar{x} = x^0 = 0.53$) that differ in their methods of preparation. Both samples of the solution-quenched co-crystals (one annealed at 123 °C for 48 h before etching) exhibited single, sharp melting endotherms indicative of homogeneous co-crystals characterised by $x = \bar{x}$. According to Fig. 4, within the experimental uncertainty, the annealing treatment does not significantly perturb the RR molecular morphology in the solution-quenched co-crystals. In the solution-crystallised sample, the fact that $\bar{x} = x_0$ merely indicates that the crystallisation was carried out to 100% conversion. Severe compositional heterogeneity can occur in solution-crystallised mixtures of PEH and PED¹⁹. However, the profile of the melting endotherm of this sample (Fig. 1) prompts us to suggest that only two phases are present: one, pure PED crystals ($x = 0$), comprising 15% to 25% of the sample, the other, co-crystals of PED and PEH characterised by $x = 0.68 \pm 0.04$. For this compositional distribution, the RR model again accounts for the observed value of S . (Note that using a value of $R^a > 0.64$ yields smaller values for S .)

In contrasting the observed values of S with the predictions of the model morphologies, the procedure used does presume compositional homogeneity, that is, x must be representative of the distribution of PEH throughout the macroscopic sample of co-crystals. In heterogeneous samples, specification of the distribution of composition is prerequisite to the extraction of molecular details from measurements of intensive parameters such as NMR second moments. (One can, for example, arrive at the observed value of S for the solution-crystallised co-crystal with the AR-110 morphology assuming that the low melting fraction (Fig. 1) has $x = 0.2$ and the remaining fraction is characterised by $x = 0.6$; $\bar{x} = 0.53$ for the entire sample.)

In conclusion, the NMR second moment data provide another perspective on the nature of the molecular morphology in polyethylene. The NMR experiments, unaffected by the overall configuration of the chains, complement the neutron scattering experiments in the sense that they selectively probe the local short-range structure. The NMR reconstruction of the mode of chain re-entry into the lamella appears to be independent of the method of sample preparation. While unequivocal data on solution-crystallised polyethylene requires compositionally homogeneous co-crystals (perhaps accessible through fractional crystallisation), these preliminary NMR results argue against theories of regular chain re-entry. Most significantly, the NMR method should be able to discriminate among less ideal models of molecular morphology, that is, two (three, ...) AR stems followed by a random trajectory, followed by another sequence of AR stems, and so on, until the entire polymer chain is incorporated in the crystal.

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Note added in proof: R. Voelkel and H. Sillescu have independently applied the NMR isotopic dilution method to PEH and observe the molecular morphology to be molecular weight dependent (manuscript in preparation).

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Soil stabilisation by cellulose xanthate

CELLULOSE XANTHATE is unusually effective in stabilising soil against attrition and deformation. At a concentration of about 0.1% in the soil it reduces erosion; at higher concentrations it can be used to bind soils to improve their mechanical strength. During an investigation of other uses for cellulose xanthate, we recently rediscovered its unusual interaction with soils. We describe here its formation and application in the laboratory, quantitatively compare it with several synthetic polymeric soil conditioners and suggest a mechanism for its action. With regard to the effectiveness of the xanthate soil treatment, its economy and the ready availability of agricultural waste cellulose, we hope to encourage further study of this interesting system.

The initial disclosure of soil stabilisation by cellulose xanthate was a series of patents by Meadows¹, followed by two brief reports by another group^{2,3}. As far as can be determined no further use of cellulose xanthate for soil treatment has been made. A number of other materials have been proposed or used for soil stabilisation, for example, strong composite structures can be made from polymers, asphalt or Portland cement⁴, in amounts of the order of 10% of the soil. At lower concentrations, various hydrophilic polymers, usually polyelectrolytes, can be used as conditioners to stabilise aggregates and improve tilth⁵. Widespread use of soil additives is cost limited. Experience in applying cellulose xanthate should determine whether it is significantly cheaper than additives based on petrochemicals or energy intensive sources.

Cellulose xanthate can easily be made from any cellulosic source that contains accessible alpha cellulose: rice or wheat straw, chemically pulped wood or paper products, cotton, and similar materials. Mechanically pulped wood or papers are not generally useful, as the structure resists solution after xantha-

tion. In this work cellulose xanthate solutions were made from used computer cards, a source of nearly pure fibrous cellulose. The cards (~2.5 g each) were soaked for 10 min in 18% (w/w) aqueous NaOH, drained and padded to dampness with paper towels. The damp alkali cellulose was transferred to a wide-mouth jar, and 1.5 ml carbon disulphide was added for each card. After rapid mixing the jar was tightly closed. Several hours later the xanthate formed an orangish sticky mass at the bottom of the jar. Using a mechanical stirrer, the xanthate was diluted to a 2% solution (based on cellulose content), which was of low enough viscosity to facilitate later measured dilutions. The shelf life of aqueous xanthate solutions is short, a few days at most, so experiments were initiated soon after preparation. Cellulose is regenerable from the xanthate solution by acidification, heating, increasing the ionic strength, or merely by waiting. In soil treatment with xanthate, hastening regeneration is generally unnecessary.

Imperial Valley clay (California) was used to test the stabilising effect of cellulose xanthate. After rough grinding to remove lumps, 20 samples of 20 g each were prepared. This clay was found to form a thick paste when 5–6 ml of water was mixed with 20 g clay. Thus, to obtain a series of samples with known cellulose content, appropriate amounts of the 2% stock solution of cellulose xanthate were diluted to about 5 ml and mixed with the clay. By this method, treatment levels of 0.01, 0.025, 0.05, 0.1, 0.25 and 0.5% cellulose (dry weight) were added to the clay. Two control samples were similarly prepared with water only. Partially dried treated clay was stirred to form irregular balls about 1 cm in diameter. After air drying and curing for a minimum of 24 h at room temperature, the aggregates were sized to pass a 2 mesh (12.7 mm) but not an 8 mesh (3.2 mm) screen. The dry weight of each sample of sized aggregates was redetermined.

To estimate relative stability, a sample was placed in a 236-ml bottle with 125 ml of water. After 1 min, the bottle was rotated end over end at 30 r.p.m. The dry weight of fines that passed through a 20 mesh (1.3 mm) screen was determined after agitation periods of 1, 4, and 16 min. Weight losses are given in Table 1.

For comparison, two synthetic polymeric soil conditioners were also tested: polyvinyl alcohol (PVA) and a hydrolysed polyacrylonitrile (HPAN). These conditioners were applied at the same treatment levels as the cellulose xanthate and tested in the same way with the rotating agitator. Results are also shown in Table 1.

Qualitative differences in texture were noted among the various treatments after drying: both the xanthate and HPAN treatments gave distinctly hardened aggregates, whereas the PVA produced more crumbly material. At higher treatment levels the xanthate-treated soil was especially firm when wet, with a rubber-like springiness. The weight loss during agitation also occurred differently among the three treatments. The rotatory test was rather harsh and, as the test progressed, xanthate-treated and to a lesser extent HPAN-treated aggregates tended to become rounded, while the PVA treatment gave irregular aggregates that gradually broke up. Weight loss of the xanthate-treated clay is, in most cases, substantially less than that of the PVA- or HPAN-treated clay (Table 1).

The mechanism of soil stabilisation by cellulose xanthate may be unique in that it seems to depend initially on adsorption as a polyelectrolyte and then later on the regeneration of cellulose. The behaviour of cellulose xanthate as a polyelectrolyte is well known⁶, as is the effectiveness of polyelectrolytes in binding together clay particles⁷. The xanthate as applied is the ionised sodium salt of the polyanion; hence, its initial interaction with soils should be largely by adsorption to the surface of the clay component. This is apparent from the immediate stabilisation of clay by xanthate, noticeable even when still wet; this rapid stabilisation does not occur with sands.

After some time, most of the xanthate is regenerated to cellulose; cellulose fibrils then presumably take the place of adsorbed xanthate to bridge between particles and confer

Table 1 Percentage weight loss of Imperial Valley clay aggregates treated with various amounts of soil stabilisers

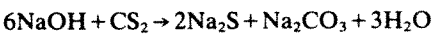
Wt % stabiliser added	1 min wash			4 min wash			16 min wash		
	Cell	PVA	HPAN	Cell	PVA	HPAN	Cell	PVA	HPAN
0 (control)	48	(48)	(48)	84	(84)	(84)	100	(100)	(100)
0.01	36	39	47	72	75	82	100	100	100
0.025	25	39	45	56	75	80	94	100	100
0.05	14	40	37	41	72	70	83	100	100
0.1	7	39	39	26	69	74	63	100	100
0.25	3	19	33	10	38	64	26	64	94
0.5	2	10	10	6	19	24	17	41	57

Aggregates and stabilisers were agitated in water for 1, 4 or 16 min after an initial 1 min static soak. Stabilisers used were cellulose xanthate (Cell), polyvinyl alcohol (PVA), and hydrolysed polyacrylonitrile (HPAN). Xanthate was prepared as described in the text. The PVA was Du Pont 'Elvanol' polyvinyl alcohol, grade 72-51, 98.5-100% hydrolysed, MW 54,000. HPAN was obtained from a sample of 'Ortho-Til' (Ortho), designated as 83% HPAN; the sample was identified as sodium polyacrylate.

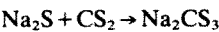
stability. Evidence for the shift from polyelectrolyte sorption in the xanthate form to binding by regenerated cellulose is afforded by analysis for oxidisable sulphur remaining in the treated samples. The analytical method was based on oxidising divalent sulphur by hydrogen peroxide to form the sulphate⁸, after which a slight excess of barium ion was added, and the decrease in soluble barium determined by atomic absorption⁹. Table 2 shows the oxidisable sulphur thus determined for a kaolin sample initially treated with cellulose xanthate to a cellulose level of 0.5%. The results are also given as weight per cent cellulose xanthate (calculated as cellulose), assuming one xanthate group for each two anhydroglucose monomer units. The initial cellulose content calculated from the sulphur content is 0.8%; the amount over 0.5% may represent sulphur present as excess CS₂. After 700 h, the oxidisable sulphur decreased to 2 mg per 100 g kaolin, representing a xanthate content of 0.01% (calculated as cellulose). As clay stabilisation by xanthate seems permanent (barring microbial action), regenerated cellulose must be the effective binding agent in the long run.

Microbial action may limit some applications. The lifetime and fate of the regenerated cellulose in practical applications are unknown, but it appears to last for at least several months³. In our laboratory, samples have been kept in water for more than six months with no marked change. Appropriate antimicrobial agents may be useful for producing a more permanent treatment.

For some applications of cellulose xanthate, its relatively short shelf life in the presence of water may be objectionable. We have found that drying at room temperature and refrigeration are effective in retarding regeneration to cellulose, although these expedients delay rather than prevent regeneration. The reason for this lies in the numerous side reactions attending xanthate formation, two of the more important being



and



The trithiocarbonate is largely responsible for the orange colour of prepared xanthate; it is a stable salt, as is the carbonate and to a lesser extent the sulphide. These reactions are rather slow¹⁰, evidently slower than xanthate formation, which is complete within a few hours at room temperature. Later, however, the reaction tends to be driven toward the formation of thio-carbonate, or at least to the sulphide/carbonate stage if CS₂ is deficient, with the cellulose xanthate decomposing to supply the necessary NaOH and CS₂, along with cellulose. Reducing the temperature slows regeneration, as does removal of water, since ionic reactions are involved. Meadows¹ found that adding a chemical dehydrating agent (for example, CaO) to the air-dried xanthate permitted stable storage for much longer times. Addi-

tional calcium ion is often beneficial for soil applications and this inexpensive addition should ameliorate the stability problem.

Spraying or sprinkling permits direct application of dilute xanthate solutions to large areas. In test plots of 1-4 m², we produced erosion resistance by applying a 0.4% cellulose solution to a depth of 1 cm, leaving a cellulose content of 0.07% in the soil. From adjacent plots tilted to about 20° angles, the silt runoff from an untreated plot was over 500 times that from a treated plot.

In test plots, treated soil appeared to absorb water more rapidly, indicating an increased permeability. This was verified

Table 2 Oxidisable sulphur content of kaolin treated with cellulose xanthate

Time (h)	Oxidisable sulphur (mg per 100 g kaolin)	% Cellulose xanthate in kaolin calculated as cellulose
0	162	0.82
0.2	149	0.75
1	124	0.63
4	123	0.62
20	83	0.42
44	70	0.35
200	36	0.18
700	2	0.01

The analytical values given for zero time were calculated from analysis of the added xanthate solution.

in the laboratory by measuring the volume absorbed by Imperial silty clay standing under a 1 cm head of water for 16 min. Samples were passed through a hand mill to obtain similar particle size and uniformly packed in 9-cm diameter cans by rapping the perimeter with a small rubber mallet. The surfaces of the untreated and bulk treated clay samples were water wetted to 1 cm depth and dried for 24 h before the test; a third sample was wetted with a dilute xanthate solution to 1 cm depth and similarly dried. Untreated clay absorbed 8 l m⁻² of exposed surface. Clay treated to contain 0.1% cellulose in the top centimetre absorbed 12 l m⁻², and clay treated to contain 0.1% cellulose throughout its bulk absorbed 19 l m⁻².

Soil treated with cellulose xanthate does not appear to affect seed germination and growth, as was observed both from weed growth on the test plots and from germination of wheat seeds in the laboratory. However, if the treated soil is compacted with a roller, a hard surface is produced that seems to inhibit seed germination.

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Sedimentation rates determined by ^{137}Cs dating in a rapidly accreting salt marsh

SEA-LEVEL records indicate that the coast of Louisiana and other parts of the Gulf Coast are rapidly subsiding¹. Louisiana is now losing approximately 16 square miles of land per year, primarily to subsidence²; the rates of subsidence vary with location. Vertical marsh accretion is the process which counteracts subsidence and eustatic sea-level rise and prevents marsh deterioration, but, as in Louisiana's salt marshes, the pattern, rate and variability are sufficiently complicated to defy simple prediction. Conditions of marsh development vary throughout the coast, from the modern and Atchafalaya deltas through the abandoned deltas to the Chenier Plain³. In recent years, much of the coastal area such as Barataria Basin has been deprived of river-borne sediment through natural stream diversion and the construction of water-control embankments. In addition, dredging from petroleum operations has altered water flow and sedimentation patterns. The survival and productivity of Gulf Coast marshes depend on the influx and accumulation of sediment that offsets the effect of subsidence and maintains the marsh surface within the tidal range. To predict long-range trends in marsh stability, accurate measurements are needed of both subsidence and sedimentation rates. Information on subsidence is available from tide gauge measurements but no measurements have been made of sedimentation rates in marshland developed on Recent Mississippi alluvium. ^{137}Cs , a fallout product of nuclear testing, has become a useful tool for dating recent sedimentary sequences in lakes⁴⁻⁸. We report here its use in the measurement of sedimentation rates in a Louisiana coastal marsh, the first report of such use in coastal marshes.

Cores were taken from a streamside and inland *Spartina alterniflora* salt marsh and from an adjoining shallow water lake in Barataria Basin in Louisiana (29°13' N, 90°7' W). From the streamside location two sediment cores were taken 7 m inland and 5 m apart parallel to a natural stream. Another set of two cores was taken 45 m inland and 5 m apart parallel to the same stream. A third set of two cores was taken 350 m apart in adjacent Airplane Lake, a shallow 19-hectare lake. Cores were obtained by twisting a thin-walled aluminium cylinder of 15 cm diameter and 53 cm depth into the marsh soil or lake bottom. Compaction was minimal using these relatively large-diameter thin-walled cylinders. The sediment was sectioned into 3-cm increments, dried and ground, and each sample well mixed.

^{137}Cs activity in each section was determined by γ counting of the oven-dried sample using a lithium-drifted germanium detector and multichannel analyser⁹. Organic carbon was determined by dry combustion. Organic matter was estimated by multiplying carbon content by 1.724 (ref. 10). Density was determined from oven-dry sediment in the known volume of each section.

Sedimentation rates were calculated from the peak ^{137}Cs concentration found in the marsh and lake profile which corresponded to 1963, the year of peak ^{137}Cs fallout, and 1954, the first year of significant ^{137}Cs fallout^{6,7}. There should be little mobility of ^{137}Cs in sediment, as it is rapidly adsorbed by clay components of sediment and soil^{8,11}. Also, the sediment captured by the dense marsh grass is unlikely to be reworked.

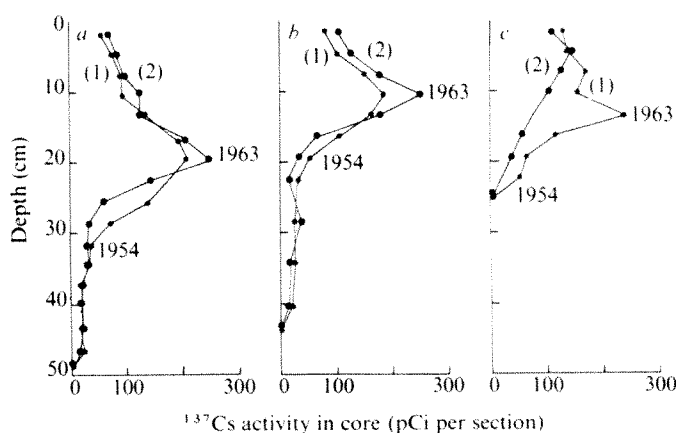
^{137}Cs profile distributions demonstrate rapid vertical marsh accretion (Fig. 1). The two marsh sites nearer the stream were found to be accreting at a rate of 1.35 cm yr⁻¹. The inland marsh was accreting at a slower rate (0.75 cm yr⁻¹). The inland marsh is beginning to deteriorate into small open water areas, presumably because its vertical accretion rate is not able to compensate for subsidence and eustatic sea-level rise. A tail of ^{137}Cs activity extended below the 1954 marker in the marsh profile but not in the lake profiles. This tail corresponds to the rooting depth of *S. alterniflora* and is probably the result of plant absorption of ^{137}Cs (ref. 10). ^{137}Cs activity was expressed on a volume (pCi per 3-cm section) basis.

The distribution of ^{137}Cs in Airplane Lake cores implies accretion rates of 1.1 cm yr⁻¹. A greater amount of sediment mixing occurred in the lake than in the marsh. One of the lake sites did not have a distinct ^{137}Cs peak. However, the 1954 marker horizon for both cores extended to the same depth. A ^{137}Cs tail was not found in the lake cores, apparently because of the absence of plant roots.

The accretion rates reported here are greater than those found in Atlantic coast salt marshes. Richard¹³ recorded accretion rates of 0.34 cm yr⁻¹ in a Long Island salt marsh using brick dust as a marker layer. Armentano and Woodwell¹⁴, using ^{210}Pb , measured accretion rates of 0.47-0.63 cm yr⁻¹ also in a Long Island marsh. The rapid accretion rates obtained by ^{137}Cs dating for this Louisiana salt marsh approximately equal reported subsidence rates of 1.29 and 1.12 cm yr⁻¹ estimated by tide gauge measurements from 1959 to 1971 in this coastal area at Bayou Rigaud (29°16' N, 89°58' W) and Eugene Island (29°22' N, 91°23' W), respectively¹. Even though there is a net land loss along the coast, vertical accretion is apparently compensating for a portion of the subsidence.

Density of the marsh soils ranged between 0.30 g cm⁻³ and 0.10 g cm⁻³, depending on depth and location (Fig. 2). Density of the lake sediment ranged from 0.45 g cm⁻³ at the surface to approximately 0.65 g cm⁻³ at 50 cm. Both mineral and organic matter contribute to the density values. From density and

Fig. 1 Measured ^{137}Cs profiles at each location. a. Streamside 1 and 2; b. inland 1 and 2; c. Airplane Lake 1 and 2.



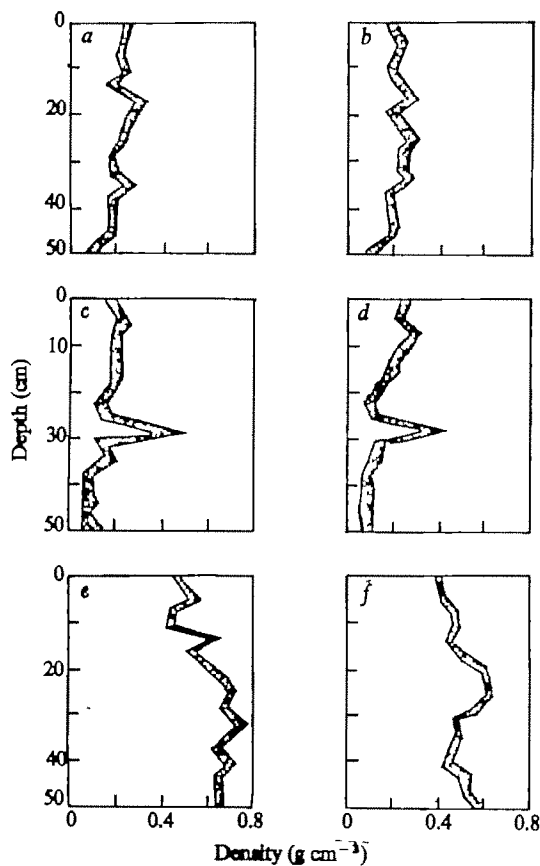


Fig. 2 Distribution of mineral and organic material at each location. a, Streamside 1; b, streamside 2; c, inland 1; d, inland 2; e, Airplane Lake 1; f, Airplane lake 2. Light shading, mineral; dark shading, organic.

carbon content of the cores, it is shown that the area vertically accretes through organic detritus accumulation and sediment input. The lake is receiving a larger amount of mineral sediment than the adjacent marsh.

The entrapment and stabilisation of suspended inorganic sediment on the marsh surface by vegetation is an important process in helping to maintain elevation with respect to sea level. The incoming sediment also supplies nutrients for plants which in turn enhance further sediment entrapment and stabilisation. The increased primary production contributes to the organic pool of these peaty marsh soils. Maintenance of a viable marsh is thus affected through aggradational processes of plant growth, organic detritus accumulation and inorganic deposition.

The ^{137}Cs dating technique thus seems to be useful in determining sedimentation rates over the past quarter-century in salt marshes that are rapidly accreting. Sediment deposited on the marsh surface is stabilised by the dense vegetation and, based on the slope of the ^{137}Cs profiles, there is little reworking and no pronounced biogenic disturbance of the deposited sediment.

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Seismological and geological aspects of the Mantaro landslide in Peru

ON the evening of April 25 1974 one of the largest landslides known took place in the Mayunmarca valley (12.6°S, 74.6°W), adjacent to the Mantaro River, in the Peruvian Andes (Fig. 1). A total of 1,000 to 1,300 $\times 10^6 \text{ m}^3$ of soil and rock was displaced in successive slides, which occurred within a few minutes of each other. Part of the slide material reached the Mantaro canyon, where a gigantic natural dam was formed. The dam was 3.8 km long extending along the axis of the canyon, 2.5 km wide across the river (Fig. 2), 170 m high from the river bed to the lowest point on the crest, and had a volume of 800 $\times 10^6 \text{ m}^3$. At the time of the slide, the Mantaro River was discharging nearly 150 $\text{m}^3 \text{ s}^{-1}$, and a reservoir was quickly impounded upstream from the dam. At its largest, the reservoir was approximately 32 km long and contained over 670 $\times 10^6 \text{ m}^3$ of water. After 42 days, the reservoir overtopped the natural dam. The high potential of flood damage was minimised by construction of a controlled spillway across the dam.

As a direct result of the slide, the village of Mayunmarca and several farms dispersed over 9.6 km^2 were destroyed. Approximately 400 people lost their lives, but about 200 more would have been killed had they not been evacuated: evacuation had been recommended in November 1973 following a geological study in which the landslide was forecast¹. Large property losses, which included a section of the main road, buildings, and agricultural fields, severely affected the weak economy of this remote region. Large sections of the main road which run parallel to the Mantaro River, were flooded by the reservoir, making the road no longer serviceable.

The Mayunmarca valley is an old glacial valley with a dendritic drainage system, which, before the slide, was formed by several streams cutting fluvial terraces into deep gullies. These streams converged near Mayunmarca and continued to the Mantaro River by the Cochacay gully (Fig. 1). Underground drainage came from the Pumarana River and from the Yanacocha and Minascocha lagoons, located at elevations >4,000 m. The hills surrounding the valley reach altitudes of >4,300 m, while the bed of the Mantaro River is just under 2,500 m in elevation. These hills are mainly formed by Permian sandstones and shales lying on Palaeozoic schist and phyllites, which are the impermeable basement rocks of the Mayunmarca valley. The sandstones and shales are generally overlain by glacial deposits or by unconsolidated, weathered, and permeable Quaternary alluvium over 100 m thick (Fig. 2).

During the geological study that warned of a possible landslide¹, an abnormally large amount of water in the unconsolidated sediments under the Mayunmarca terrace and signs of recent small slides in the flanks of the surrounding hills were noticed. After the landslide, a geological inspection of the area²⁻⁴ revealed water saturation of the unconsolidated sediments and soils, suggesting loss of cohesion of the upper unconsolidated layers resulting from a rise in the water table and an increase in underground flow. This condition aided in triggering the slide and was helped by the steep gradient of the Mayunmarca valley.

The landslide area has had very low seismic activity in the past 70 yr. Only two seismic events were located in this area, both

being of intermediate depth of focus ($h \sim 100$ km). Local and regional seismic activity during the few days before the slide, as recorded at Huancayo (HUA, $\Delta = 82$ km, $AZ = 327^\circ$) and Ñaña (NNA, $\Delta = 240$ km, $AZ = 284^\circ$), consisted of four small earthquakes that occurred >100 km from HUA, and of a magnitude $m_b = 5.8$ earthquake that occurred approximately 660 km southeast of Mayunmarca and about 17 h before the landslide⁵. Earth tremors were reported near the Mayunmarca valley between 03:00 and 04:00 hours and around 21:00 hours

LT on 25 April. The earlier tremor could correspond to the $m_b = 5.8$ regional event, which occurred 660 km away from Mayunmarca, and the later tremor, to the landslide itself. These possibilities suggest that the landslide was not caused by a seismic event. This hypothesis is supported by the appearance of the short-period seismograms from the landslide and recorded at HUA, NNA, and Arequipa (ARE, $\Delta = 581$ km, $AZ = 138^\circ$), which are similar to the records obtained at Pasadena, California, from slumps originating during petroleum drilling opera-

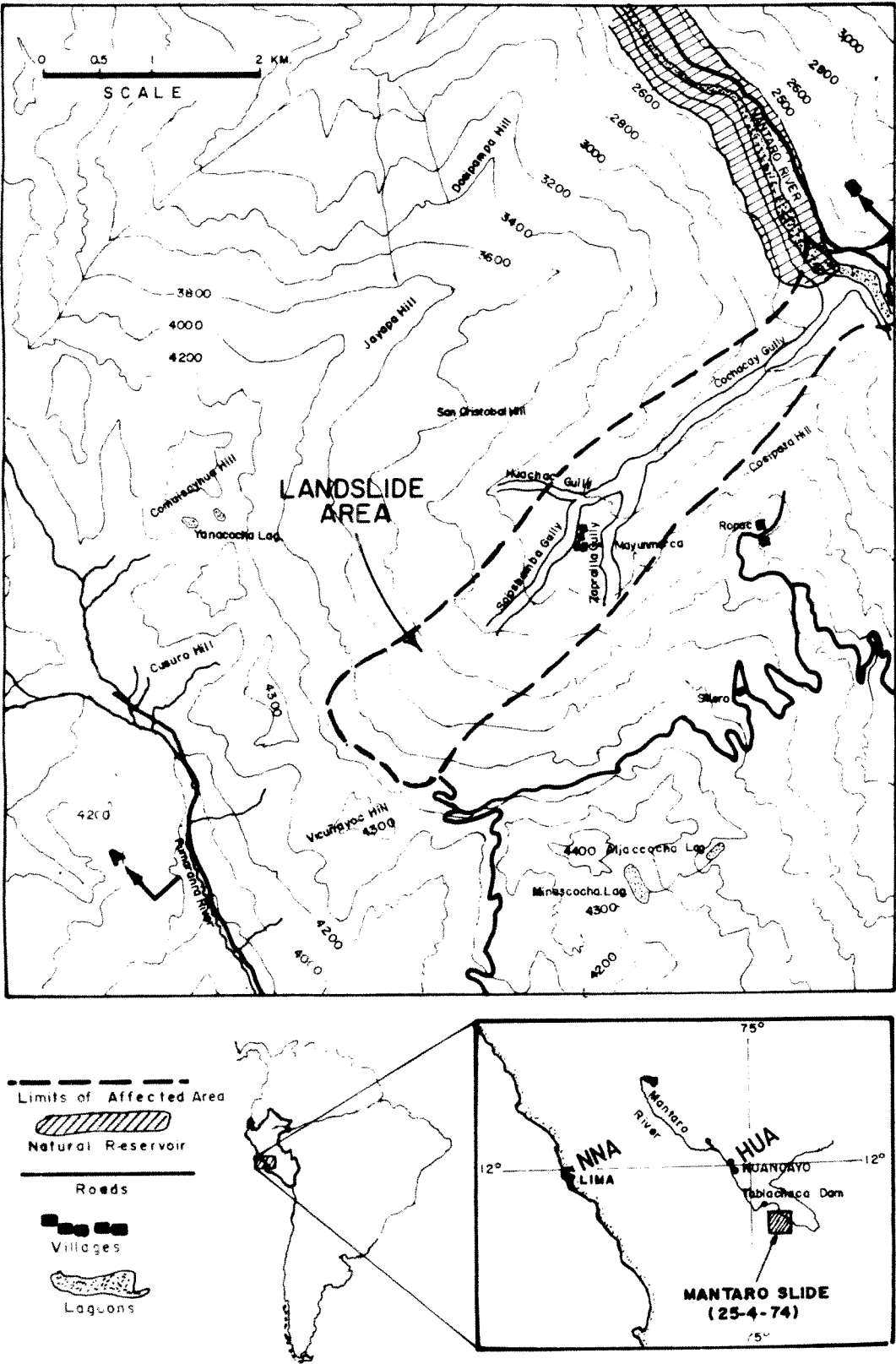
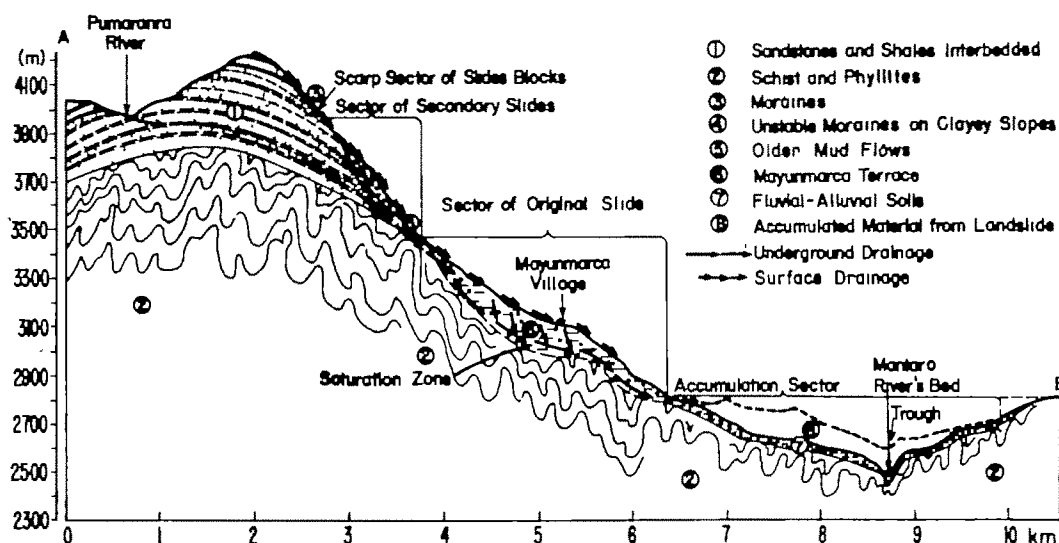


Fig. 1 Location map of the Mayunmarca Valley showing the principal surrounding topographical features (in m) and the area affected by the 25 April 1974, landslide. A-B location of cross section shown in Fig. 2.

Fig. 2 Section A-B of the Mayunmarca Valley (see Fig. 1) showing major geological and topographical features related with the landslide of 25 April 1974. Vertical scale = $2.5 \times$ horizontal scale.



tions near Terminal Islands⁷. However, the possibility has not been eliminated that a small earthquake, whose magnitude was so small that it was not recorded at the local seismic stations, was the triggering source of the landslide. A seismic survey by the Instituto Geofísico del Perú^{5,6} some weeks after the landslide showed the existence of microearthquakes of possible tectonic origin; they had epicentral distances between 2 and 20 km from the recording site, which was located 20 km from the debris dam and at the upstream end of the reservoir and 3 km from the Tablachaca concrete dam (Fig. 1), which serves the Mantaro Hydroelectric Plant. These microearthquakes could have been associated with the presence of the Tablachaca reservoir or the reservoir created by the Mantaro landslide. A special seismic study could be used to determine the origin of this micro-earthquake activity and to determine whether a fault suggested from surface tectonic features and coinciding with the Mayunmarca valley is active or not.

Seismic waves generated by the Mantaro landslide were recorded at several South American observatories as far away as 2,890 km (ref. 8). Short- and long-period seismograms were recorded at HUA, NNA, and ARE. Only long-period signals were recorded at Las Peñas (PNS, $\Delta \sim 900$ km) and at Brasília (BDF, $\Delta = 2,890$ km).

The origin time of the landslide was 0157 22.5 UTC on 26 April 1974 (2057 22.5 LT on 25 April), calculated from P-arrival times on the HUA short-period seismograms and using Herrin's seismological tables⁹. The disturbance recorded on the HUA and NNA short-period seismograms lasted 4 min, while long-period records show higher frequency waves arriving 3 min after the onset.

From the seismic phases originated by the Mantaro landslide (long-period signals shown in Fig. 3), the geological and physiological constitution of the Mayunmarca valley before the event¹, and the vertical distribution of different types of debris in the natural dam², we infer that the landslide was a complex phenomenon composed of different slides which occurred during a period no longer than 4 min but longer than $1\frac{1}{2}$ min. The first slide consisted of unconsolidated soils and sediments underlying the Mayunmarca terrace, nearly $3.5 \times 1.6 \times 0.1$ km (560×10^6 m³). After this original slide, the material in the flanks of the surrounding hills became unstable; therefore, most of the remaining material (around 700×10^6 m³) slid in subsequent secondary slides on the top of the affected area and of the debris belonging to the original slide. Large blocks of rocks on top of the surrounding hills also became unstable and fell; some of this material rolled along the disturbed area and reached the natural dam (7 km away) formed by the previous slides.

A large-amplitude phase arriving 70 s after the onset, which was observed on the HUA and NNA short-period seismograms,

points to the impact of the original slide material on the Mantaro canyon, 3.5 km from the Mayunmarca terrace's middle point. This arrival time suggests an average mass-displacement velocity of 180 km h^{-1} .

The kinetic energy developed during the landslide, using an average slide velocity of 180 km h^{-1} , a material density of 2.7 g cm^{-3} , and a total volume of $1,300 \times 10^6 \text{ m}^3$, was about 4.4×10^{22} erg. Using the same physical dimensions as those of the material involved in the landslide, and the distances shown in Fig. 2, the potential gravitational energy of the Mantaro landslide is of the order of 10^{24} erg.

The energy radiated as seismic energy, E , was calculated from local-station seismograms using the relation¹⁰:

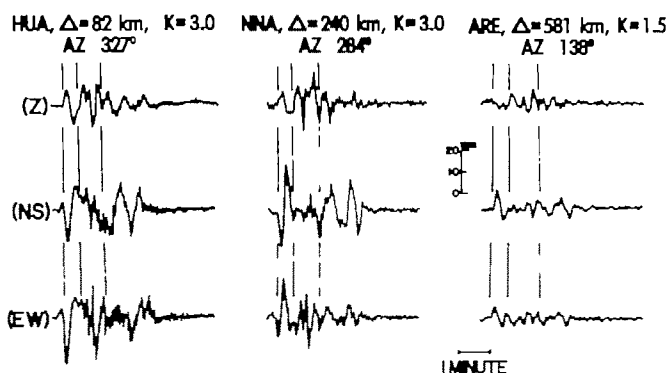
$$\log E = 11.72 + 1.68 M_s \quad (1)$$

The magnitude values, M_s , were calculated from standard equations¹¹.

The values for M_s and E that correspond to the maximum amplitude phases generated by the Mantaro landslide are 3.6 and 6.3×10^{17} erg, respectively, from long-period records, and 3.9 and 18.7×10^{17} erg, respectively, from short-period seismograms.

The M_s value obtained from a BDF long-period seismogram is 4.1, yields an elastic energy of 8.9×10^{17} erg, as computed from the Gutenberg-Richter¹² energy-magnitude relationship. This value is in agreement with the energy values obtained from local-station seismograms.

Fig. 3 Long-period seismograms of the landslide of 25 April 1974 recorded at Huancayo (HUA), Ñaña (NNA), and Arequipa (ARE), showing three seismic phases produced by the landslide. Δ = epicentral distance (km), K , magnification $\times 10^3$, and AZ = azimuth in degrees.



The above results show that seismic energy generated by the 1974 Mantaro landslide was of the order of 10^{18} erg, with a corresponding $M_s = 4.0$. Therefore, the radiated seismic energy is about four orders of magnitude less (0.01%) than the kinetic energy developed during the landslide, which in turn is about two orders of magnitude less (1.0%) than the potential gravitational energy.

The Mantaro landslide is one of the first large landslides to have been widely recorded by several seismic observatories, at local and teleseismic distances. These recordings made calculation of some of the landslide's physical parameters possible. A complete analysis of the seismograms and a detailed interpretation of the landslide mechanism, assessed from such analysis, is in progress.

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Volcanics from
the Sierra Leone Rise

THE Sierra Leone Rise, located in the east equatorial Atlantic, forms a discontinuous chain of seamounts as shallow as 2 km extending with a general NE–SW trend from near the Sierra Leone coast of Africa, to the St Paul fracture zone near the Mid-Atlantic Ridge (Fig. 1). The origin of this feature has remained a topic of discussion. Sheridan *et al.*¹ have hypothesised that the Sierra Leone Rise is a volcanic structure formed at the beginning of the opening of the Atlantic in the early Cretaceous period. The twin features of the Sierra Leone and the Ceara Rises are probably of oceanic origin and were created 80 Myr ago or later in their present-day position with respect to Africa and South America². The Atlantic ocean exhibits several similar aseismic structures which appear symmetrically oriented with respect to the mid-oceanic ridge, such as the Walvis–Rio Grande Rise and the Iceland Faeroes–Iceland Greenland Ridges. These structures are volcanic edifices having a composition similar to that found in their associated islands^{3–7}. Deep sea drilling of the Ceara Rise^{8,9} penetrated a basaltic basement of the upper Cretaceous period (Maestrichtian) (Leg 39, Site 354). Similarly, a DSDP hole (Leg 41, Site 366) on the Sierra Leone

Rise, penetrated sediments of the same period, without reaching basement¹⁰. We report here the discovery of alkali-rich volcanics in an area of the Sierra Leone Rise. The sediment overlying the rock fragments is aged ~45 Myr.

During the Romancha cruise of the RV Jean Charcot in December 1977 we studied the south eastern slopes of the Sierra Leone Rise, near DSDP Site 366, with the objective of sampling crustal material (Fig. 1). From a detailed bathymetric survey, using a multichannel narrow beam echo-sounder, it was possible to locate the best existing outcrops for dredging. However, despite this, our dredging operation proved unsuccessful, mainly because of the topography of the area surveyed, which consists of gently rolling sediment-covered hills having slopes of <10°.

A sediment core (1.6 m long) was taken in the same area (lat 05°14.9' N; long 20°15.1' W) as the site of the dredging (Fig. 1). This core was recovered from a depth of 2,720 m near the summit of a seamount, which appeared to be the site of important erosional processes resulting from bottom currents (Fig. 2). This core penetrated a sediment veneer and entered a highly altered basement. The upper levels in the core (0–67 cm) are upper Pleistocene to Holocene in age (NN21–N23) with extensive reworked material as old as middle Eocene, especially from 25 to 67 cm. Despite some disparity between the nannofossil and foraminiferal record, only the Oligocene appears absent. From 67 to 110 cm, the ages range from upper to middle

Table 1 Chemical analyses of material collected from a piston core (CH 77-07) from the Sierra Leone Rise

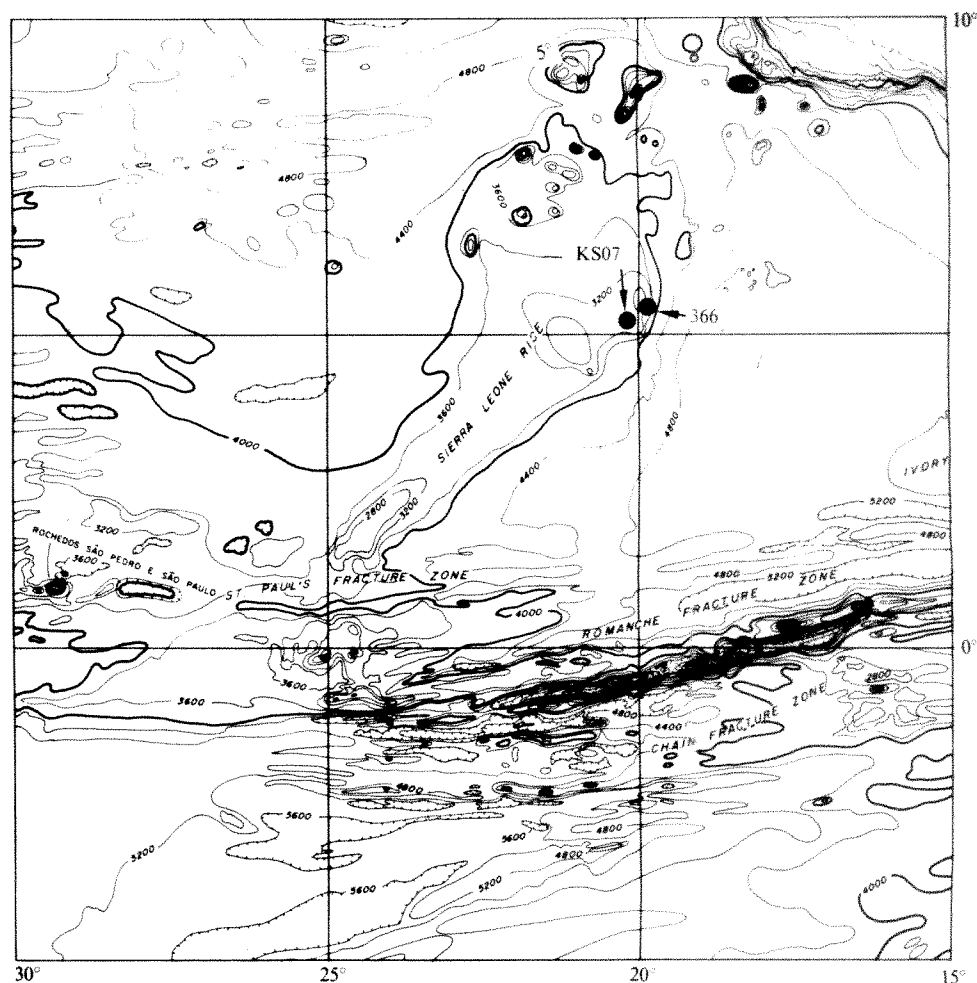
	Clay-like fraction (wt %)	Feldspar in rock fragment	
		Phenocryst	Microphenocryst
SiO ₂	40.95	59.7	64.6
Al ₂ O ₃	11.46	25.7	22.4
Total Fe as FeO	7.73	0.24	0.3
MnO	0.16	—	—
MgO	2.91	—	—
CaO	3.93	7.7	3.1
Na ₂ O	4.03	6.4	9.0
K ₂ O	2.76	0.15	0.6
TiO ₂	1.94	—	—
P ₂ O ₅	2.03	—	—
H ₂ O	15.38	—	—
H ₂ O ⁺	6.12	—	—
Total	99.4	99.89	100.0
Molecular composition	—	or _{1.9} ab _{56.7} an _{41.4}	or _{7.3} ab _{76.6} an _{16.1}

The small clay-like section matrix made up mainly of smectite and zeolite was analysed by X-ray fluorescence (P. Cambon and J. Etou-bleau). The minerals were determined by energy dispersive analysis of X rays.

Eocene (about 45 Myr BP) with some upper and middle Paleocene (60 Myr BP) foraminifera observed. From 110 to 130 cm, the sediment is a foraminiferal chalk epigenised to apatite. The biogenic elements include fish remains and unidentified casts of foraminifera. Below 130 cm no biogenic elements are observed.

The change in mineralogy corresponds to the stratigraphical limits encountered in the core: from 0 to 67 cm depth in the core, the known carbonate fraction comprising about 15% of bulk material consists mainly of kaolinite with some smectite and quartz. At 67–110 cm the smectites forming the main constituents of this level are associated with well-crystallised zeolite of the heulandite type. This latter material is volcanic in origin. Apatite occurs in the lower section of the core at 110 cm. The contact below 130 cm is made up of a thin (<0.3 cm) iron manganese lamina overlying a green brecciated clay-rich material. This material consists mainly of soft green clay aggregates intermixed with reddish brown abundantly altered and highly vesicular rock fragments, and scattered grains of clino-

Fig. 1 Bathymetric map of the Sierra Leone Rise from Uchupi¹⁴ showing the location of the core CH 77-07 and of the Deep Sea Drilling hole 366¹⁰.



pyroxene and feldspar with sharp crystalline boundaries. The small fraction is formed of well-crystallised smectite, and two types of zeolites, heulandite and phillipsite, are also present in smaller amounts. This small clay-like fraction has a mean composition relatively high in silica (41%), and a high total alkali content ($\text{Na}_2\text{O} = 4\%$, $\text{K}_2\text{O} = 2.8\%$) (Table 1), which is compatible with the mineralogy. The relatively high P_2O_5 content (2.0%) reflects the presence of apatite needles.

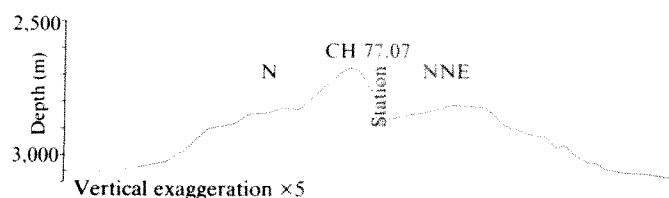
The type of mineral association (pyroxene, feldspar, apatite, smectite and zeolite) is also encountered in the rock fragments (~3 cm diameter) found within the material recovered. These rock fragments consist of early-formed feldspar set in an altered ferruginous matrix containing pyrite, apatite and tiny laths of parallel oriented feldspar indicating a trachytic textural feature. The feldspars in the rocks are tabular with faint twinning and do not show any signs of secondary replacements. The groundmass is reddish brown due to the extensive alteration, with iron oxide material and smectite being the main alteration minerals encountered. Scanning electron microscope observations show that the zeolites developed *in situ* in geode-like structures. Because of the extended degree of alteration in the groundmass, the chemical analyses of the latter did not give any indication of the nature of the rock. However, microprobe (EDAX) analyses of the unaltered feldspar suggest the alkaline character of the samples.

The composition of the feldspar is indicated in Table 1 and shown on a ternary diagram (or-ab-an) in Fig. 3. The potassic and the sodic ($\text{or}_{1.9} \text{ab}_{56.7} \text{an}_{41.4}$ — $\text{or}_{7.3} \text{ab}_{76.6} \text{an}_{16.1}$) nature of the feldspathic phases differs considerably from that found on the accreting plate boundary of the Mid-Atlantic Ridge (Fig. 3). The molecular percentage of orthoclase in the feldspar from the MAR basalts is insignificant^{11,12} when compared with that of the

Sierra Leone Rise. However, there is a similarity in the composition of the Sierra Leone Rise (Fig. 3) feldspar to the alkaline volcanic series from oceanic islands (such as Hawaii, Saint Helena and Gough islands) (Fig. 3). In addition, the mineral composition and the textural features of the rocks from the Sierra Leone Rise suggest the trachyandesite type of volcanism encountered on the Walvis Ridge⁴. The feldspar analysed from the Walvis Ridge alkali basalt (sample WD 3-4) is also of a potassic nature ($\text{or}_{40} \text{ab}_{45.9} \text{an}_{50.1}$ — $\text{or}_{2.4} \text{ab}_{28.6} \text{an}_{69.0}$) like that from the Sierra Leone Rise (Fig. 3).

In conclusion, both the study of the clay fraction and the rock fragments demonstrate that we have sampled an extremely weathered volcanic outcrop near the top of one of the seamounts of the Sierra Leone Rise. This outcrop is covered by a thin intensely reworked carbonate ooze including Eocene to Palaeocene fauna, overlying a phosphate epigenised foraminiferal chalk of unknown age. This suggests that the last eruptive phase recorded in the sampling area occurred 45 Myr BP, and gave rise to fractionated volcanics of an alkaline nature.

Fig. 2 A profile drawn from a multichannel narrow beam survey chart made in the sampled area located near station CH 77-07 on the Sierra Leone Rise.



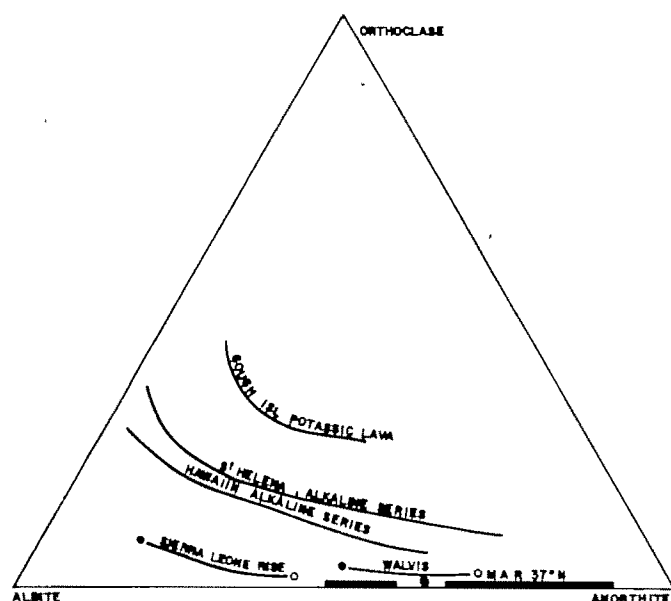


Fig. 3 An-or-ab ternary diagram of feldspar composition from various localities. The data from Gough Island¹⁵, from Hawaii¹⁶ and from St Helena¹⁷ we compared with that from the Walvis Ridge and the Sierra Leone Rise. An open circle indicates phenocrysts of analysed feldspar and the filled circles are matrix feldspar found in the corresponding rock samples. The data on the plagioclase from near 37° N in the Atlantic ocean were taken from basalts of the inner floor¹¹ and those from the Rift Mountain Region¹² of the Rift Valley.

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An ophiolite complex of probable early Caledonian age discovered on Karmøy

THE existence, in the Lower Palaeozoic sequence of western Norway, of a major ophiolite complex which is outlined here is shown best on the island of Karmøy, western Norway. Our preliminary interpretation is that the ophiolite complex was thrust into position during the Grampian phase of the Caledonian orogeny, although it has probably been subsequently displaced during the end-Silurian phase of that orogeny.

It is clearly established that the allochthonous Caledonian sequence of western Norway consists of two major complexes, with different tectono-metamorphic evolution, separated by a major stratigraphic unconformity of middle/upper Ordovician age¹⁻³. The older complex shows the more involved tectono-metamorphic history and in many areas was subjected to strong retrogression during the end-Silurian phase of the Caledonian orogeny. The main metamorphism and deformation of this older complex has been correlated with the Grampian deformation in the Scottish and Irish Dalradian^{3,9}. Within the older sequence, thick piles of submarine metabasalts are widely developed. They show pronounced geochemical similarities to ocean-floor basalts, and have been presumed to relate to ridge spreading in a proto-Atlantic ocean⁴⁻⁷. The metabasaltic sequences may be of considerable thickness and in many areas are associated with intrusive cumulate gabbros, trondhjemites and minor developments of serpentinised peridotites. This has given rise to speculations on the possible ophiolitic affinities of the assemblage⁸. However, none of the previously described sequences demonstrate a fully developed ophiolite stratigraphy. Indeed, in many areas, such as the Bergen Arcs^{7,9}, the thick submarine metabasalts and their associated intrusive rocks lie stratigraphically above a considerable thickness of metasediments.

Our recent investigations in the Karmøy region have revealed the presence of a cross section through a major early Caledonian ophiolite complex, which we call the Karmøy Ophiolite. The basal contact of the ophiolite is exposed on the Haugesund peninsula and represents a low angle thrust. On Karmøy the ophiolite sequence is truncated by a major stratigraphic unconformity (Figs 1 and 2) beneath the Skudeneshet Group of middle/upper Ordovician age. The ophiolite contains a tripartite stratigraphy in ascending order as follows.

Zone III: a lowermost plutonic zone comprising layered gabbroic and ultrabasic intrusions together with minor developments of trondhjemites, and cut by later basic dykes. The gabbroic and ultrabasic rocks represent a polyphasic series of intrusions and are in many cases well banded cumulates. Observations of structures in the cumulate sequence show consistent upward younging. These rocks and the associated trondhjemitic intrusions contain evidence of successive phases of high temperature deformation before the emplacement of a major linear steeply inclined dyke swarm of metabasalts and diabases which extends into the overlying submarine metabasaltic pile. This pattern, of pre-dyke deformation is analogous with described occurrences from the Oman ophiolite¹⁰, and the Bay of Islands ophiolite¹¹⁻¹³. At the top of this plutonic zone the dyke swarm becomes a sheeted dyke complex, well exposed at the Airport, and we refer to this as the Lufthavnen sheeted dyke complex. This attains in places 100% dyke rock with only minor disconnected screens of wall rock. These screens are of gabbro in the lower part of the sheeted dyke complex. Screens of gabbro are not found in the upper parts, but gradually it becomes possible, as the sheeted dykes become less dense, to distinguish screens of pillowed metabasalt. In places, however, the contact with the overlying pillow sequence is complicated by later faulting. The dyke swarm observed in the plutonic zone increases in density up towards the true sheeted dyke complex.

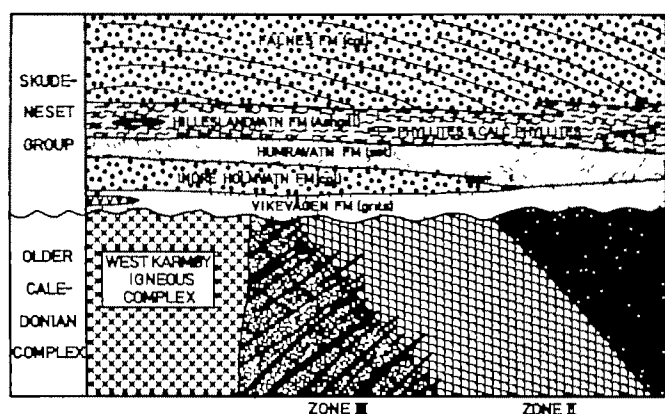
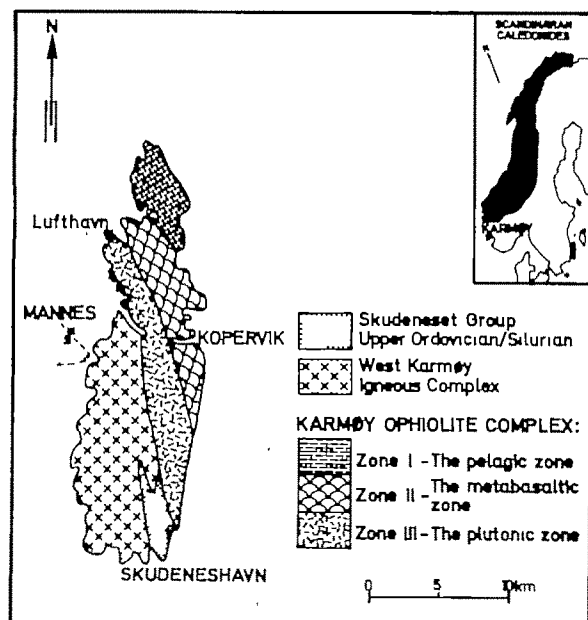


Fig. 1 Simplified geological map of Karmøy showing distribution of major units.

Zone II: above this a >2 km thick sequence of greenstones occurs which contains both pillowed and massive flow units of metabasalt, with irregularly developed hyaloclastites. The rocks within this zone show varying intensities of deformation, ultimately producing greenschists from the basalts, although where way-up structures have been observed they show consistent younging away from the lower plutonic zone III. In the upper part of the metabasalt column, horizons of greenschists, which may represent volcanoclastic materials, and irregular jasper layers and bedded cherts are intercalated with metabasalts. Dyke rocks similar to those observed in zone III also cut the metabasalt sequence, but diminish in intensity upwards. Several examples have been observed where such dykes feed individual flow units, and we conclude that the dyke swarm represents the feeders to the lava pile.

Zone I: overlying the metabasalts of zone II, and preserved in a large secondary synform, there occurs a sequence, several hundred metres thick, consisting of ribbon cherts, bedded jasperites, black schists, greenschists which may be of volcanoclastic origin, minor calcareous horizons and in the lower part, thin flow units of pillowed basalts. The main schistosity is related to upright F_1 folds with subhorizontal northwesterly trending axis and an associated steeply dipping northwesterly

Fig. 2 Idealised profile illustrating the relationships of the Karmøy Ophiolite; the rocks of the Skudeneshet Group, though folded, are drawn horizontal. In zone III, nos 1-4 indicate the polyphasic nature of the plutonic assemblage.



trending axial planar schistosity. The rocks of zone I are considered to represent a series of pelagic sediments.

We consider that the zonal sequence I-III described above represents, respectively, layers 1, 2 and 3 of a typical cross-section through oceanic crust. Massive sulphide deposits (Cu, Ni) occur on Karmøy and the neighbouring small islands, though as yet no podiform chromites have been observed in the serpentinised peridotites.

It is not possible, at this stage to provide a precise age for the thrusting (obduction) of the Karmøy Ophiolite on to the Baltic Shield, although by analogy with other Caledonian ophiolite complexes in the North Atlantic region, that is, the Girvan¹⁴ and Newfoundland complexes¹⁴, it may be of lower Ordovician age. The Karmøy Ophiolite, having been both deformed and metamorphosed in greenschist facies, was then intruded by the rocks of the West Karmøy Igneous Complex¹⁵. This latter is a late syntectonic complex of granitic and dioritic rocks with at least five major phases of intrusion. Preliminary Rb/Sr whole rock isochron studies indicate that late granodioritic dykes have an age of ~450 Myr ($\lambda^{87}\text{Rb} = 1.39 \times 10^{-11} \text{ yr}^{-1}$) with an initial $^{87}\text{Sr}/^{86}\text{Sr}$ of 0.7210. The obduction of the Karmøy Ophiolite, on to the Baltic Shield, is considered to have occurred before the emplacement of the West Karmøy Igneous Complex, and thus, the date obtained for the granodioritic dykes provides a minimum age for ophiolite obduction. The presence of highly assimilated gneiss xenoliths in some of the granitic rocks, and the high initial $^{87}\text{Sr}/^{86}\text{Sr}$ ratio of the granodioritic dykes, implies a continental crustal source for the West Karmøy Igneous Complex, beneath the ophiolite. It is thus speculated that the granitic and dioritic magmas originated through partial melting in the downgoing continental plate of Baltic Shield crystallines, perhaps in relation to the active translation of the ophiolite slab.

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The source of chert for aboriginal artefacts in southwestern Australia

ABORIGINAL chert artefacts abound in surface assemblages along the western margin of southwestern Australia north of Perth, but no local source of chert is known. The decrease in proportion of chert in assemblages further from the coast has been used as evidence of a westerly source on the present continental shelf but no data have been provided to show that this source is practical. Recent drilling by oil companies offshore in the northern Perth Basin has now shown that chert occurs at depths within reach of the last glacial sea level low and that the postulated source is not only reasonable but highly probable.

Chert is favoured over other materials because of its hardness, and conchoidal fracture which make working of edges and

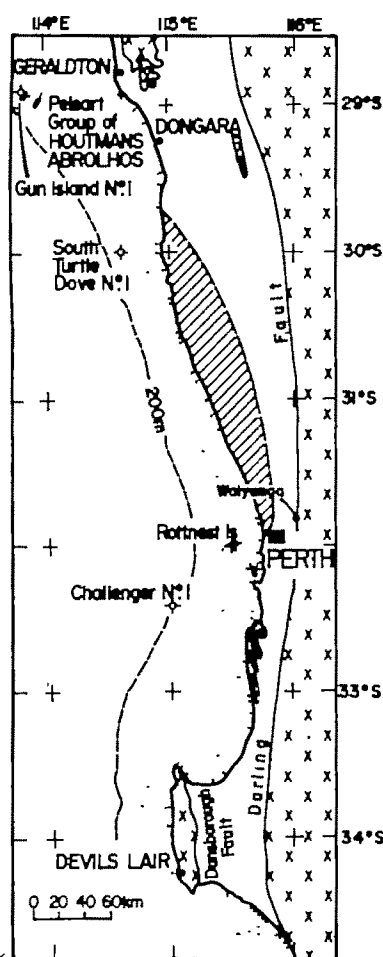


Fig. 1 Occurrence of Eocene chert artefacts (cross hatched area) in relation to the Precambrian Shield area (crosses) in southwestern Australia.

points simple, and because the products are durable. Several recent papers¹⁻⁶ have discussed the distribution of the artefacts including the aspect of source because no similar chert is known in outcrop in the Perth Basin. Glover and Cockbain¹ showed that the chert is of Middle and/or Late Eocene age on the basis of the characteristic benthic foraminiferid *Mastinella chapmani* Glaessner and Wade.

Chert, identical to that found north of Perth, occurs as artefacts in sediment dated at 12,000–17,000 yr BP from the excavations at Devils Lair on the southwestern tip of Western Australia² and more recently³, chert as recent as perhaps 4,500 yr BP has been discovered at Walyunga, close to Perth. The former age in particular corresponds closely with the last glacially induced sea level low estimated at about 105 m below modern sea level⁷, after allowing for hydroisostatic depression of the continental margin. New data have become available from examination of sediments from four wells offshore in the Perth Basin (Fig. 1)—Challenger No. 1 (WAPET, 1975)⁸, Rottneest Island Bore (W.A. Govt. 1913)⁹, South Turtle Dove No. 1 (WAPET, 1975) and particularly Gun Island No. 1 (BP-1968)⁹—whose Cainozoic stratigraphic sections are shown on Fig. 2. Challenger No. 1 and Gun Island No. 1 both contain Late Eocene sections of cherty limestone of P15/P16^{10,11} age sediments, that from Gun Island also containing *Mastinella chapmani*. Rottneest Island Bore contains a Middle Eocene section^{8,12,13} without chert but South Turtle Dove has Middle Eocene limestone with chert equivalent in age to sediments in the Rottneest Island Bore. The Middle Eocene sediments in the Rottneest Island Bore and South Turtle Dove No. 1 represent deposition during a Middle Eocene interval of high sea level, a

different transgression from the Late Eocene¹⁰ sea level high indicated by the sediments in the other two wells and elsewhere⁸. Thus the most likely source of artefacts, if outcrop occurred, would be the Late Eocene unit, although Middle Eocene cherty limestone cannot be ruled out as an additional source.

The most significant section is that encountered in Gun Island No. 1. The uppermost sample⁹ recovered from this well was a core (Core No. 1) which contains an excellently preserved fauna of Late Eocene planktonic foraminiferids, including *Globigerinatheka index index* (Finlay), *Globorotalia cerroazulensis* (Cole), *Globigerina tapuiensis* Blow and Banner, *G. gortanii praeturritina* Blow and Banner, *G. utilitindex* Jenkins and Orr, and *Pseudoglobobulina primitiva* (Finlay) which indicate a Late Eocene age (P15/P16)¹¹. This material was originally identified as Late Miocene in age⁹. It occurs at 131 m below sea level and probably extends higher. The base of the Late Eocene section is at 228 or 252 m below sea level, the doubt hinging on the age of two undated samples at 246 and 252 m below sea level. Somewhere above the Eocene section is some Miocene,

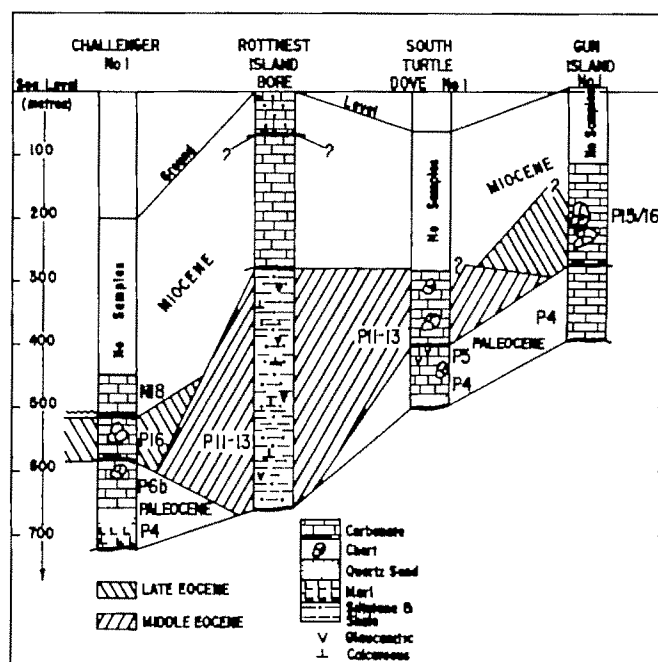


Fig. 2 Perth Basin sections with Middle-Late Eocene rocks.

required to explain the presence of *Lepidocyclina* in cutting samples from deeper in the well.

In consequence of the discovery of Middle-Late Eocene cherty sediments in several localities west of the present coastline, and at depths within reach of known Quaternary sea level depressions, it is clear that outcrops of these sediments occurred on the present inner continental shelf during the last glacial sea level low and that these provided the source of chert for aboriginal man's artefacts. A westerly source is also indicated by the higher proportion of chert in artefact collections taken from near the present coastline, the proportion decreasing eastward². A westerly source has been suggested^{4,8} and the results of drilling Challenger No. 1 reported⁸, but the results from Gun Island No. 1 are much more relevant to the problem and convincing in proving the source. Searching the continental shelf of Australia and elsewhere will reveal other evidence of prehistoric man's presence. As the Eocene is recognised as an epoch whose sediments commonly contain chert¹⁴, it behoves scientists working on passive continental margins, particularly adjacent to areas of low rainfall and runoff, such as southwest Africa, to be aware of the possibility of the outcrop of Eocene chert during

intervals of low sea level, and of finding evidence of prehistoric human habitation on the present shelf during times of low sea level.

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Why do we not perceive photons?

VERY few photons need to be captured by the receptors of the human eye to excite a sensation of light. The number required is certainly no greater than 100 and may be much less^{1,2}. A single rod requires only one photon to signal light³. A question which therefore arises is why do we not see punctate photon absorptions when we look at a very dim patch of light? The retina catches incident photons only sporadically in time, and therefore in space, when a source is very dim. If very few photons are sufficient to detect light, it might be expected that we should see isolated flashes of light, so that a patch of light appears mottled or speckled. Indeed, if a dim object is viewed on the screen of a cascaded image intensifier, so that each quantum caught by the cathode is amplified, we do see quanta as individual flashes or specks on the display phosphor². This apparent contradiction creates a physiological problem ignored in most standard textbooks on vision although the issue has been raised^{4,5}. We report here a possible solution; we have observed point sources of light set up one at a time very rapidly and at random by computer within a square patch on the face of an oscilloscope and find that as the luminance of the point sources is decreased the number of specks seen increases markedly, and that the appearance of motion, due presumably in the first place to the effect of the chance sequences of specks on motion-sensitive neurones, becomes weaker. We propose that at or near the threshold, the number of specks seen increases to infinity so that the specks crowd together to appear uniform and so the motion ceases.

To test this, after becoming adapted to the dark, we viewed the oscilloscope through a neutral density wedge. Although persistence on the oscilloscope phosphor is brief, a collection of points of changing distribution but constant mean number appears continuously in view. This reflects the operation of storage mechanisms in vision which prolong the visibility of each speck. The number of specks visible to the observer provides a measure of the time constants involved. In a dark room the wedge filter was first set so that nothing was visible. Then it was adjusted until the shape of the square patch on the screen was just continuously discernible. At this level of luminance it was also possible to identify correctly dark shapes (a circle, a square or a triangle, each of which was one-quarter the area of the basic

square patch) in front of the screen. The display looked uniform and devoid of motion, although no more than one light point was being displayed on the screen at any instant.

Now a uniform patch was generated filling the area where the point display had been. A switch enabled us to interchange the point and uniform display. At the threshold for shape identification, the uniform patch could not be distinguished from the random point display.

If the luminance of the point display was again increased, mottled blobs were seen moving around sluggishly; no individual points could be resolved. The display then looked quite different from the uniform display at any luminance. A further small increase in luminance changed the appearance of the patch to a very large number of small discrete points each agitatedly jumping about. The transition from mottled blobs to sharp discrete points occurred over a small range of luminance. As the luminance is raised further to very high levels, the number of specks perceived decreases, each speck appears brighter, and movement becomes less agitated.

These changes in number, motion and texture are so dramatic that a naive observer is astonished that nothing about the display has changed except the luminance, over which he has personal control.

In further experiment, points were plotted at random in a rectangular patch, but every few seconds, four points, arranged to form a square, were displayed, each once only, alongside the rectangular patch. The four points were each of the same luminance as those plotted at random within the rectangular patch. As the luminance decreased the isolated four points were visible only sporadically. Sometimes all four might be seen, sometimes none were seen, and sometimes a random subset was seen. The rectangular patch was seen as uniform only if we could never see any one of the four isolated points.

This finding implies that points are additive in their effects at the luminance levels where the patch presents a uniform appearance. Individual points on their own emit too few photons ever to be visible as specks. Persistence of individual points, perceived as specks, therefore does not explain the impression of uniformity: another effect is involved. A clue to this additional effect is provided by close observation of the four isolated points near threshold. Each point, if visible at all, varies on each appearance, in size, from a small spot to a large globule, and in orientation, sometimes being elongated along one axis, sometimes along another.

Pirenne and Denton³ suggested that at the threshold for vision, the number of quanta required for a sensation of light may be the same for detectors which respond to the activity of different groups of receptors. If so, it will be those detectors with a greater catchment network of receptors which will be most likely to respond when the light is least. It is also these same detectors which are most likely to respond to quanta caught from two or more point sources in different places, as they encompass a larger number of receptors. The functional organisation of the retina will seem to change⁶, when, in reality, all that has happened is that certain detectors are now favoured by the circumstance of occasional quantum emission.

Considerable evidence, both psychophysical and neurophysiological, suggests that vision is organised into channels⁷ specialised for size, and, independently, orientation⁸. Our observation of variation in appearance of single points of light near threshold is consistent with the assumption that sometimes one, and sometimes another, of such channels are by chance obtaining a sufficient quantum catch to report on the appearance of a single spot of light. An analogous effect of variable appearance in the orientation of short lines was reported by Andrews⁹. Those channels which report a larger size are, of course, favoured.

The appearance of uniformity both of a uniform surface in clear light and of a surface made of kinetic points appearing at random, seems to be the combined result of two effects. One is a prolongation of the impression made by quanta caught in either case. The other is a consequence of the organisation of the visual

system into detectors arranged to catch larger patterns of light as the number of quanta caught becomes more sparse. It is perhaps fortunate that our visual system has been so designed as to present uniform surfaces to us as uniform at these low photon levels. If this were not so, surfaces would dissolve in the night into collections of scintillating spots, and the shower of photons from the dim night-sky would obscure the perception of the star formations.

An incidental observation was that any of the four individual points in the last experiment could seem to vary in depth, as well as in size and orientation, when observed with both eyes. This effect is presumably due to random stimulation of different disparity detectors at these low light levels.

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On feeding on more than one trophic level

IN trying to understand the structure of ecological communities, ecologists usually pay particular attention to the interactions between pairs, or small groups of species¹. Questions about the 'shape' of the food webs within which these species are embedded are much more rarely asked²⁻⁴. For example, what happens when a population feeds at more than one trophic level (omnivory)? In some real food webs there seem to be no omnivores (Fig. 1a)⁵; in others omnivores are common^{6,7} (Fig. 1c)⁸. In this note we attack the problem of omnivory using simple, linear Lotka-Volterra models of food webs⁹, and show that certain patterns are much more likely to persist on an evolutionary time scale than others. We then compare the model predictions with real food webs.

Consider a system of n interacting species described by equations of the form

$$\frac{dX_i}{dt} = X_i \left(b_i + \sum_{j=1}^n a_{ij} X_j \right) \quad (1)$$

where X_i is the population size and b_i the instantaneous rate of growth of the i th species, and a_{ij} is the *per capita* effect of the j th species on the i th species. We used equation (1) to construct model food webs, using as our criterion for realism the notion that ecologically feasible models must be locally stable^{1,3,9}, and that a rapid return to equilibrium enhances stability^{9,10}.

For models with four species and four trophic levels there are eight possible configurations (Fig. 2). These are distinguished by the 'rank of omnivory', which is the number of predator-prey interactions in excess of those in the model with no omnivory, and by the position of these interactions. Matrices, with sign configurations corresponding to these models were analysed

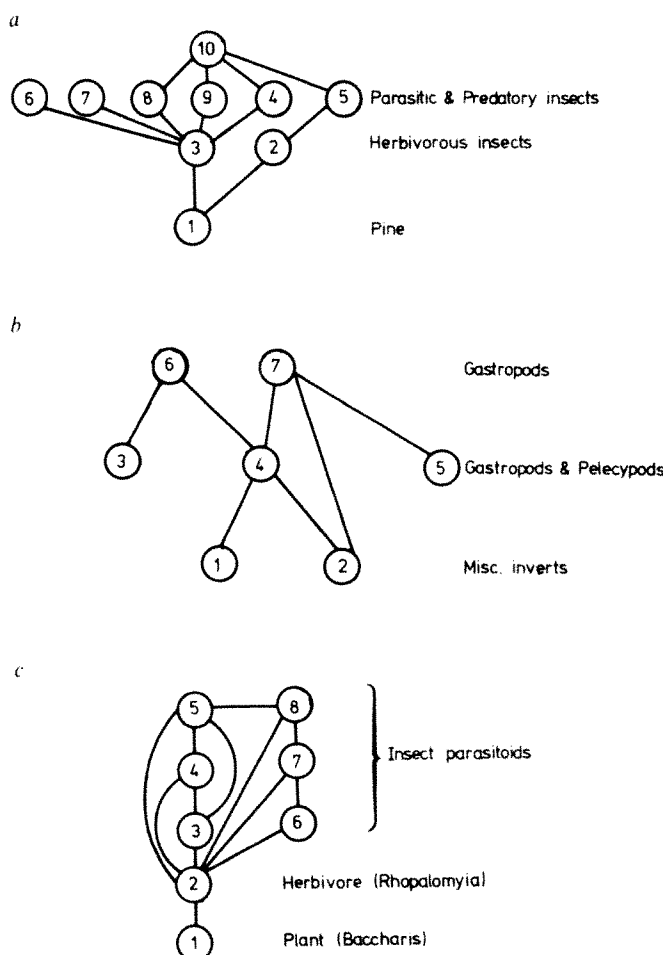
numerically to determine their maximum eigenvalues ($\lambda_{\max}$), their stability [$\text{Real}(\lambda_{\max}) < 0$] and their relative return times to equilibrium, RT , defined by

$$RT \equiv -\frac{1}{\text{Real}(\lambda_{\max})} \quad \lambda_{\max} < 0$$

The elements of the matrices were chosen uniformly at random with 1,000 replications over three sets of parameter limits, labelled 'vertebrate', 'vertebrate/insect', and 'parasitoid', respectively.

(1) Vertebrate: The off-diagonal elements of the matrices are the products of the *per capita* effect of the prey on the predator multiplied by the predator's equilibrium density or the *per capita* effect of the predator on the prey multiplied by the prey's equilibrium density ($a_{ij}X_i^*$). When predators are larger than their prey the effect of the predator on the prey will be much larger than the converse and the predator's density will be smaller than that of its prey. The elements corresponding to the predator's effect on the prey will therefore be of much greater absolute value than the converse; we chose values on the interval of $(-10, 0)$ for the former and $(0, +0.1)$ for the latter for this set of models. (Note that very large, invertebrate predators (such as starfish) and their prey will also fall into this category.)

Fig. 1 Diagrammatic representation of three natural food webs. *a*, Relationships between insects feeding on Pine⁵. *b*, A simplified representation of the interactions between intertidal gastropods, pelecypods and their prey⁶. Interactions accounting for less than 20% of the predator's diet have been omitted; without this simplification omnivory is common in this web. *c*, Part of a simplified insect host-parasitoid web on a single food plant⁸. Omnivory is common in such webs.



(2) Vertebrate/insect: The *per capita* effect of an insect feeding on a tree is very small, the converse very large. For this set of simulations we chose the interval of the effect of trophic level one on two to be (0, +10) and the converse to be (-0.1, 0). The remainder of the interactions were as (1).

(3) Parasitoid: Here, interactions between trophic levels one and two were as in (2). The remainder were chosen to mimic insect host-parasitoid interactions¹¹, in which the equilibrium population sizes of host and parasitoid may be more similar than in (1), and the *per capita* effect of the host on the parasitoid is large (one host frequently gives rise to one parasitoid). The reciprocal interactions were therefore assigned similar magnitudes, on the intervals: parasitoid on host (-1, 0), host on parasitoid (0, +1).

In all the models, the diagonal elements for the heterotrophic species were zero, implying that each species in the chain (except the first) was ultimately limited only by the abundance of food in the preceding trophic level and not, for example, by behavioral conventions^{12,13}. The species at the base of each food chain (plants) were self limited, with coefficients distributed on the interval (-1, 0).

The option of choosing Jacobian entries directly over the more frequent choice of the b_i and the a_{ij} has several advantages. Rates of increase and decrease in the absence of all other species (b_i) are very difficult for us to visualise; the equilibrium populations (X_i^*) and the effects of predators on prey and vice versa (a_{ij}) are much more obvious. Our models necessarily have positive X_i^* , as the Jacobian entries and the a_{ij} have exactly the same signs. Thus we do not need to calculate the X_i^* from the b_i and the a_{ij} , check their positivity and then calculate the Jacobian; the savings in computation are quite considerable.

For the three sets of eight models, the percentage of unstable models and those stable models with return times in excess of 100 are shown in Table 1. Note first that as the rank of omnivory increases, the percentage of unstable models increases, but for the subsets that are stable, the percentage with long return times generally decreases. These effects work in opposition to one another, so that in the real world, feasibility could either increase or decrease with rank. However, for the vertebrate and vertebrate/insect models, very few matrices were stable for rank greater than one. Second, models of rank one differ in their feasibility, depending on how many levels are spanned by the predator. While over 9% of the vertebrate and vertebrate/insect models are stable when the predator feeds on levels one and two, or two and three, less than 4% of the models are

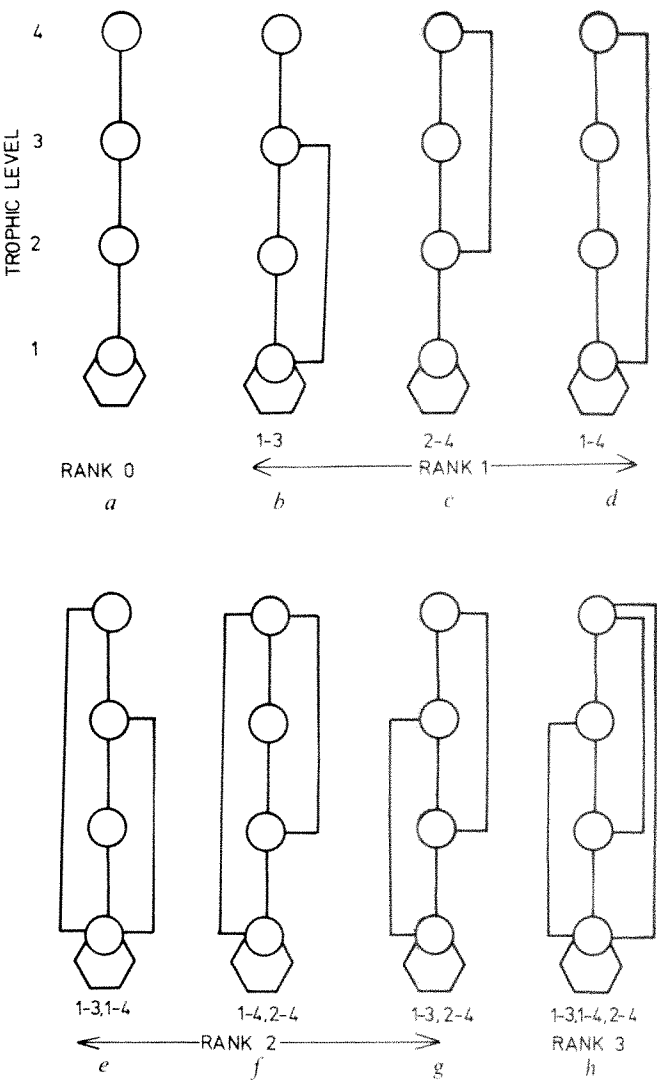


Fig. 2 Theoretical food-chains. *a*, Without omnivores; *b-d*, with one omnivore-link; *e-g*, with two and *h*, with three omnivore links. The species at the base of each food chain (plants, level 1) are self-limiting. These food chains can be modelled using equations of the form specified by equation (1).

Table 1 Stability of 1,000 random food webs of each type, constructed according to the range of parameter values specified in the text

Percentages of unstable models				
Rank of omnivory	Position (species linked)	Vertebrate	Models Vertebrate/insect	Parasitoid
0	—	0	0	0
1	1-3	81	78	40
	2-4	91	91	68
	1-4	96	96	51
2	1-4 & 2-4	98	98	59
	1-3 & 2-4	97	97	74
	1-4 & 1-3	99	99	50
3	1-3, 1-4, & 2-4	100	100	72
Percentages of stable models with return times > 100				
0	—	44	41	42
1	1-3	21	22	19
	2-4	11	13	19
	1-4	47	46	38
2	1-4 & 2-4	—	—	11
	1-3 & 2-4	—	—	13
	1-4 & 1-3	—	—	13
3	1-3, 2-4, 1-4	—	—	13

Percentages are given to the nearest integer.

stable where a predator feeds on levels one and three. In these latter models a high percentage also have long return times. Third and last, although vertebrate and vertebrate/insect webs show almost identical patterns, the parasitoid webs are generally much more stable though their return times are comparable. High ranking omnivory is relatively easily attained in these webs.

Increasing the rank of omnivory in these models also increases connectance with its well known effect on decreasing stability. However, it is important to investigate the precise restrictions on web shape that low connectance implies. We show elsewhere¹⁴ that our results still hold if the effects of increasing omnivory and connectance are changed independently.

Bringing these results together, we predict that webs with large numbers of omnivores (high ranking omnivory) will be rare in the real world, except in insect host-parasitoid systems (or other groups of species with comparable interaction coefficients and equilibrium population sizes). We also predict that it will be extremely rare to find species that feed simultaneously both high and low in a food web.

Using data on 19 food webs from tropical, temperate, aquatic and terrestrial systems accumulated by Cohen¹⁵ we examined 58 food chains with three or more trophic levels. Of these, 24 had no omnivores, 23 omnivory of rank one, and 11 of rank two or

more. At first sight, 11 webs with high-ranking omnivory is more than might be expected on the basis of our models. However, an examination of the 11 webs showed that in three cases the omnivory was associated with gross lumping of categories (invertebrates, insects and zooplankton), and hence is probably an artefact of the data (because species that are otherwise similar but which feed at different trophic levels are grouped together). Consideration of the remaining webs also highlights a more fundamental problem—the difficulty of distinguishing between links in real food webs that significantly depress the populations being fed upon (controlling links, as in the models) from those that do not. Inclusion of the latter inevitably leads to omnivory (and connectance in general³) being overestimated. This may be compensated for in part by discounting links that provide only a small percentage of an omnivore's diet. Thus, omitting links that make up less than 20% of the prey items taken by omnivores in Paine's⁶ study of a very complex intertidal community, yields a simplified web with only one omnivore (Fig. 1b). Similar results are obtained for the other communities studied by Paine¹⁶. Unfortunately, there are no data available which permit us to analyse the remaining food webs with high ranking omnivory in the same way, and we cannot tell what proportion of the omnivorous links in these webs is also important.

These difficulties aside, our other predictions seem to be supported by the data. Thus, of the 23 chains with omnivory of rank one, only one involves a species which feeds on trophic-levels one (plant) and three (animal) (as in Fig. 2d). The remainder feed on consecutive trophic levels, either one and two (Fig. 2b) or two and three (Fig. 2c). This distribution is significantly different from random ($P < 0.01$), and supports our second prediction. [Of the 65 omnivores examined, 30 fed on levels two and three (both animal) and 35 fed on levels one and two, suggesting that dissimilarity in diet alone is not sufficient to explain the rarity of 1–3 links.]

Only one of Cohen's webs involves insects⁵, and it was one of the simplest analysed (Fig. 1a). However, several highly complex insect-parasitoid webs have also been reported^{7,8}, and it is at least encouraging to see very complex webs developing in the real world in exactly those situations which our models predict are feasible. Figure 1c shows an example⁸. Even after making allowance for the fact that some of the links are minor, such webs tend to be more complex than nonparasitoid webs. Facultative hyperparasitism (omnivory) is particularly common in this group of animals^{7,17,18}.

Obviously there are weaknesses and omissions in most of the published data on food webs, and there are reasons other than population dynamics making omnivory difficult. For example, some species must be prevented from feeding at more than one trophic level by the markedly dissimilar types of food found in each, and some of the differences that we have observed between vertebrate and parasitoid webs may arise because of the peculiar mode of nutrition adopted by insect parasitoids. Comparisons between vertebrate and insect webs are also biased by the tendency for vertebrate ecologists to exclude true parasites from consideration. (When a fox eats a rabbit, it also eats the rabbit's parasites.) This is an unfortunate omission, because internal parasites influence the dynamics of their hosts far more than has generally been realised^{19,20}.

Omnivory in the way that we have modelled it can be generated by several different biological processes: by individuals which seek out prey from different trophic levels at one stage in their life history (conventional omnivory); by different age-classes of one species feeding on different foods (or hosts in the case of true parasites (R. M. Anderson, personal communication)) and by individuals consuming prey and its internal parasites 'in the same mouthful'. But irrespective of the mechanism by which they are generated, trophic links must satisfy certain dynamical constraints if the resulting food webs are to persist. Our models suggest that some trophic patterns and combinations of parameter values are much more stable than others. We believe that considerations of this kind may have an important, but neglected role in determining whether

particular species or whole sets of species can coexist in ecological systems, and points the way to a more general understanding of the design of natural food webs.

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Competition among desert perennials

THE accurate description of horizontal patterns of distribution and the determination of the mechanisms resulting in these patterns^{1,2} are primary objectives of plant ecology. Although there have been many studies of horizontal patterns, most investigations have not proceeded beyond the initial stage of detection and analysis of pattern, leaving suggestions of causation untested. Controlled field experiments that test mechanisms which may regulate distributions are rare³. We have designed a controlled field experiment to evaluate the effects of intra- and interspecific interactions affecting two distinct horizontal patterns, thereby providing a test of current plant distribution theory.

We selected a 2.5-hectare area in the Mojave Desert near Lucerne Valley, San Bernardino County, California (34°33' N, 116°53' W). The terrain is level, ensuring even deposition of rainfall, and consists of deep granitic alluvium. The vegetation is co-dominated by *Larrea tridentata* Cav. (Zygophyllaceae) and *Ambrosia dumosa* (Gray) Payne (Asteraceae) with derived species' importance values (based on their relative dominance, relative frequency and relative density in 104 5 m × 5 m random quadrats) of 39.7% and 49.2%, respectively.

Both these species are important components of many desert communities—co-dominating 70 per cent of the Mojave Desert⁴. *Ambrosia* is short, hemispherically shaped and drought-deciduous, whereas *Larrea* is tall, cone-shaped and evergreen. Also, *Larrea* has been shown to be regularly distributed in certain areas of both the Mojave and Sonoran Deserts^{5,6}, where competition for moisture is hypothetically very keen.

To determine the horizontal distribution of these species, we sampled the site and analysed our data in a manner similar to that of Woodell *et al.*⁵. The density of *Larrea* and *Ambrosia* were measured in 250 10 m × 10 m quadrats from a random,

Table 1 Horizontal distribution of shrubs

Species	Mean ($\bar{x}$) (shrubs per 100 m ²)	Variance (σ^2)	$\frac{\sigma^2}{\bar{x}}$	Distribution
<i>Larrea tridentata</i>	3.62	2.06	0.57	Regular
<i>Ambrosia dumosa</i>	19.52	96.56	4.95	Contagious

Both species' distributions are significantly nonrandom at the 0.1% level using Student's *t* test.

stratified sample. Utilising the variance/mean ratio method described by Greig-Smith¹ (significantly >1 = clumped, significantly <1 = regular), we determined the direction and degree of non randomness. The results in Table 1 clearly indicate that the horizontal distribution of *Ambrosia* is clumped and that of *Larrea* is regular. Using Pearson product-moment linear correlations of the density and the total biomass per 5 m $\times$ 5 m quadrat of *Ambrosia* on *Larrea* (which proved insignificant), we concluded that *Larrea* and *Ambrosia* were independently distributed relative to each other⁷.

As all regular and some clumped distributions have been attributed to plant-plant interactions and because the literature suggests the occurrence of competition for moisture in the desert^{2,5,8,9,10}, we decided to investigate these possible distributional control mechanisms using a differential removal experiment in the field. Forty 100²-m circular plots (5.64 m radius) within the 2.5-hectare area were circumscribed using average-sized *Larrea* bushes for centre points and as monitored plants. From these plots four categories (10 replicates each) of control and test plots were randomly chosen. These categories were: (1) control—no plants removed; (2) total removal—all *Larrea* and *Ambrosia* except centre *Larrea* removed; (3) *Larrea* removal—all *Larrea* except centre *Larrea* removed; (4) *Ambrosia* removal—all *Ambrosia* removed. Plants were removed by cutting at ground level. We also set out four equivalent categories of plots in which individuals of *Ambrosia* were similarly utilised as both centre points for circumscribing and for monitoring. On completion of the various treatments, predawn measurements of plant water potential were taken to evaluate the water status of the monitored plants using the Scholander pressure bomb method^{11,12}.

Immediately after a 60-mm rainfall in August, we began to measure water potentials at semi-monthly intervals. Initially the water potentials of *Larrea* were ~ -20 bar and those of *Ambrosia* ~ -2 bar with no consistent differences among treatments. (These are high potentials for both species.) As drying of the soil continued and plant water potentials decreased through time, consistent differences among treatments developed (Table 2). In the *Larrea*-monitored plots the mean water potential of the monitored *Larrea* plants in the control

plots (-39.0 bar) was significantly lower than that of the *Larrea* plants in the total removal (-33.7 bar) and in the *Ambrosia* removal plots (-34.6 bar), but not significantly different from the mean water potential of the monitored plants in the *Larrea* removal plots (-39.3 bar). Similar results were obtained in the *Ambrosia*-monitored plots. The mean water potential of the monitored *Ambrosia* plants in the control plots (-44.2 bar) was significantly lower than that in the total removal plots (-30.5 bar) and in the *Larrea* removal plots (-36.5 bar), but not significantly different from the mean water potential of the monitored plants in the *Ambrosia* removal plots (-44.6 bar).

To our knowledge this is the first experimental evaluation of plant competition in deserts. The results of this study suggest that competition for soil moisture does occur at this site when water availability is low and that, with the present horizontal arrangement of individuals, interspecific competition is more intense than intraspecific competition. The actual mechanism(s) responsible for the interspecific effects on plant water potentials is undetermined. While we suspect the mechanism may be a simple depletion of a limiting resource (water) by interacting species, the possibility of allelopathy, especially by the roots, cannot be ruled out.

Furthermore, it must be emphasised that, because of the feedback type of relationship between competition and distribution, the present status of competition at this desert site may not be indicative of the historical interactions among the plants which have resulted in the present distributions. It is possible that at our site *Larrea* plants were once clumped and competed intensely with each other. Through time, such competition may have resulted in the present regular distribution in which few plants are close to each other, and competition is thereby minimised. Alternatively, *Ambrosia* plants may have remained clumped because most of their growth occurs at relatively high water potentials (> -30 bar) when water may not be a limiting resource.

The negative interactions between *Larrea* and *Ambrosia*, indicated by the plant water potentials in this study, are surprising in view of the independent distributions of these species relative to each other. These distributions may be the result of other, overriding, physiological and environmental factors, or the interspecific interactions have not been of sufficient intensity or duration to affect the distributions. The significance of the negative interactions between *Larrea* and *Ambrosia* is yet to be determined in terms of production.

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Table 2 Differential effects of removal treatments on plant water potentials

<i>Larrea</i>		<i>Ambrosia</i>	
Treatment	Water potential (bar)	Treatment	Water potential (bar)
<i>a</i> Control	-39.0 ± 1.3 ^{b,d}	<i>a</i> Control	-44.2 ± 3.2 ^{b,d}
<i>b</i> Total removal	-33.7 ± 1.6 ^{a,c}	<i>b</i> Total removal	-30.5 ± 3.4 ^{a,c,d}
<i>c</i> <i>Larrea</i> removal	-39.3 ± 2.3 ^{b,d}	<i>c</i> <i>Ambrosia</i> removal	-44.6 ± 3.9 ^{b,d}
<i>d</i> <i>Ambrosia</i> removal	-34.6 ± 2.7 ^{a,c}	<i>d</i> <i>Larrea</i> removal	-36.5 ± 2.8 ^{a,b,c}

Small letters in data column denote statistical differences from the treatments (a = control; b = total removal; c = intraspecific removal; d = interspecific removal) at $P < 0.05$ using Student's *t* test. The water potential data (14 November 1977) are based on five randomly chosen plants (three twigs per plant) per treatment.

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Diverse modes of action of biotic and abiotic phytoalexin elicitors

PHYTOALEXINS are inducibly formed higher plant metabolites that are antibiotic to certain potential plant pathogens¹. At least 75 plant species representing 20 families have been shown to accumulate phytoalexins in response to infection¹⁻³. Phytoalexins also accumulate in plants in response to various agents termed elicitors¹, including substances of pathogen origin (biotic elicitors) and abiotic elicitors such as heavy metal salts and detergents¹⁻³. Elicitors may be useful for investigation of the molecular basis of phytoalexin production or disease resistance expression¹. However, the mechanisms by which such diverse elicitor molecules induce phytoalexin accumulation in plants are unknown. I have found⁴ that levels of glyceollin, a phytoalexin produced by soybean [*Glycine max* (L.) Merr.] hypocotyls in response to infection with the fungal pathogen *Phytophthora megasperma* var. *sojae* A. A. Hildb., are regulated by relative rates of induced biosynthesis and constitutive degrading activity. I report here the effects of various biotic and abiotic elicitors on biosynthesis and degradation of glyceollin in soybean tissues.

Detached cotyledons from 8-d-old seedlings of the soybean cultivar Harosoy 63 were wounded as described by Ayers *et al.*⁵, but wounds were made on the lower surface of the cotyledons. A 80 µl drop of aqueous biotic or abiotic elicitor solution containing rifampicin and ampicillin at 10 and 500 µg ml⁻¹, respectively, was placed on to the wounded areas of 10 cotyledons and these were incubated on moist filter papers in Petri dishes at 25°C. Glyceollin did not accumulate in the control (H₂O-treated) cotyledons (Fig. 1a) despite the fact that significant levels of biosynthetic activity were present ~10 h after wounding (Fig. 1b). Failure of the control cotyledons to accumulate glyceollin seemed to be due to the presence of glyceollin degrading activity (Fig. 1c). Thus there was apparently a balance between synthesis and degradation, such that glyceollin did not accumulate. It would therefore be expected that glyceollin accumulation could be elicited by treatments that either inhibited its degradation and/or enhanced its synthesis to exceed the rate of degradation.

Treatment of the cotyledons with a biotic elicitor such as cell walls of *Phytophthora megasperma* var. *sojae*, a fungus highly pathogenic to certain cultivars of soybean¹, greatly stimulated the glyceollin biosynthetic activity of cotyledons (Fig. 1b) and resulted in considerable accumulation (Fig. 1a). The biotic elicitor, however, had no significant effect on glyceollin degrading activity (Fig. 1c). In contrast, an abiotic elicitor such as HgCl₂ also elicited glyceollin accumulation (Fig. 1a), but strongly inhibited glyceollin degrading activity of the cotyledons (Fig. 1c) with only slight effects on biosynthetic activity (Fig. 1b). These results show that the primary mechanisms of action of the biotic and abiotic elicitors are distinctly different.

All the effective abiotic elicitors of glyceollin accumulation tested, including various metals and detergents, strongly inhibited glyceollin degrading activity but their effects on synthetic activity were relatively small (Table 1). Some abiotic elicitors such as CuSO₄ and K₂Cr₂O₇ partially inhibited synthetic activity and glyceollin accumulation in response to these abiotic elicitors was reduced relative to those that did not inhibit synthetic activity (Table 1). High concentrations (10 mM) of some abiotic elicitors such as HgCl₂ and CdCl₂ did not induce glyceollin accumulation, possibly because of their high toxicity to plant cells at these concentrations as indicated by strong inhibitory effects on synthetic activity. Chemicals such as CoCl₂ and CrCl₃ that inhibited synthetic activity more strongly than degrading activity or those such as CsCl, SnCl₄ and low concentrations (0.1 mM) of HgCl₂ and CdCl₂, that had no significant effect on either synthetic or degrading activity were also inactive as elicitors of glyceollin accumulation. Thus, only those chemicals that selectively inhibited glyceollin degrading activity but not its synthetic activity were active abiotic elicitors.

In contrast to abiotic elicitors, none of the tested biotic elicitors of fungus origin inhibited glyceollin degrading activity, but all stimulated its synthetic activity to ~seven to eight times that in the control cotyledons (Table 1). Stimulation of the synthetic activity seemed to be caused only by biotic substances with elicitor activity, since soybean cell walls or any of the other biotic substances listed in Table 1 did not significantly affect glyceollin synthesis or accumulation. Thus the probable mechanism of action of biotic elicitors is stimulation of glyceollin synthesis to rates exceeding its rate of degradation.

Although the mechanisms underlying accumulation of phytoalexins by biotic elicitors are not understood¹, my earlier observations^{4,6-8} indicate that glyceollin accumulation in response to inoculation of soybeans with incompatible races of *Phytophthora megasperma* var. *sojae* may be due to increased biosynthesis resulting from *de novo* transcription of DNA. The present study indicates that such enhanced *de novo* biosynthesis may also occur in soybean after treatment with biotic elicitors, but not abiotic elicitors. The initial 'recognition' of fungal biotic elicitors by soybean cells is being studied in several laboratories^{1,5,9,10}.

The unique response of soybean cotyledons to abiotic elicitors is significant because it offers insight into the frequently observed accumulation of plant phytoalexins in response to 'stress', that is a change in the normal physiological state of the plant cell environment. The present data clearly indicate that phytoalexin accumulation can occur in response to mechanical wounding of plant tissue in conjunction with inhibition of the normal, apparently constitutive phytoalexin degrading system.

The lack of specificity of the phytoalexin response to various abiotic elicitors has been used as an argument against a role of phytoalexins in disease resistance¹. My results, however, suggest that this nonspecific phytoalexin response should be dis-

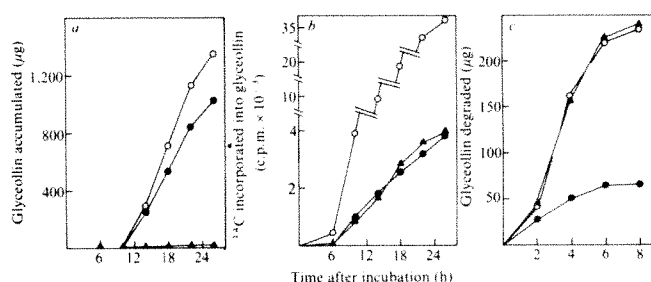


Fig. 1 Effects of ○, a biotic elicitor [Fungal cell wall (FCW), 50 µg ml⁻¹] and ●, an abiotic elicitor [HgCl₂, 1.5 mM] on accumulation (a), synthesis (b) and degradation (c) of glyceollin in soybean cotyledons. For measurement of glyceollin accumulation, the wound droplets on the elicitor-treated cotyledons were collected by elution with water at various times after incubation, and glyceollin was extracted by ethylacetate⁵ and quantitated by a TLC/UV method⁶. For estimation of glyceollin biosynthetic activity in the elicitor-treated cotyledons, each wound droplet was replaced by 10 µl of ¹⁴C-L-phenylalanine solution (15 µCi ml⁻¹, 522 mCi mmol⁻¹) at various times after incubation as indicated on the abscissa and the isotope was fed for subsequent 1 h. The wounded sides (~1 mm thick) of 10 cotyledons were then collected immediately and rates of ¹⁴C incorporation into glyceollin were determined by modifications of the TLC/UV method⁶ as described elsewhere⁴. For measurement of glyceollin degrading activity, eight freshly-collected cotyledons were sliced into halves parallel to their upper and lower surfaces and incubated in 50 ml flasks containing 10 ml of water, 300 µg of purified glyceollin, antibiotics as above, and a biotic or abiotic elicitor at the indicated concentrations. The flasks were shaken at 60 strokes per min at 25°C for various times and glyceollin remaining undegraded in the flasks was extracted by homogenisation with 20 ml of ethanol and quantitated⁶. FCW was prepared from *Phytophthora megasperma* var. *sojae* grown in a soybean hypocotyl medium⁶ for 5 d by the method of Ayers *et al.*⁹ but fungal homogenate was prepared by pulverising mycelium under liquid N₂ in a mortar and suspending in 0.5 M potassium phosphate (pH 7.2) followed by a brief sonication (1 min, 5 times). The concentration of the biotic elicitor is based on glucose equivalent by anthrone assay. ▲, Controls (water only).

Table 1 Effects of biotic and abiotic elicitors on accumulation, synthesis, and degradation of glyceollin in soybean cotyledons

Elicitor	Elicitor concentration	Accumulation (μg)	Glyceollin synthesis (% of control)	Degradation (% of control)
H ₂ O (control)	—	<20	100 (3,400–4,200 c.p.m.)*	100 (220–245 μg)*
<i>Effective abiotic elicitors</i>				
HgCl ₂	1.5 mM	972 $\pm$ 202	110 $\pm$ 34	28 $\pm$ 8
CdCl ₂	1.0 mM	932 $\pm$ 219	138	48
AgNO ₃	3.3 mM	1,280 $\pm$ 274	202 $\pm$ 56	19 $\pm$ 10
CuSO ₄	10.0 mM	472 $\pm$ 125	35	17
K ₂ Cr ₂ O ₇	3.3 mM	495 $\pm$ 194	42	29
Triton X-100	5.0 mg ml ⁻¹	1,391 $\pm$ 320	105 $\pm$ 10	28 $\pm$ 6
Nonidet P-40	5.0 mg ml ⁻¹	1,205 $\pm$ 295	98 $\pm$ 5	25
<i>Ineffective abiotic elicitors</i>				
HgCl ₂	0.1 mM	<20	119	92
	10.0 mM	<20	6	11
CdCl ₂	0.1 mM	<50	105	97
	10.0 mM	<100	9	28
CoCl ₂ †	10.0 mM	<100	28	55
CrCl ₃ †	10.0 mM	<20	18	48
CsCl†	10.0 mM	<30	139	97
SnCl ₄ †	10.0 mM	<20	113	104
<i>Effective biotic elicitors</i>				
Fungal extracellular metabolite	5.0 μg ml ⁻¹	1,352 $\pm$ 281	691 $\pm$ 181	102 $\pm$ 2
	20.0 μg ml ⁻¹	1,605 $\pm$ 309	831 $\pm$ 274	103 $\pm$ 5
Fungal cell wall	50.0 μg ml ⁻¹	1,295 $\pm$ 382	756 $\pm$ 126	97 $\pm$ 5
	250.0 μg ml ⁻¹	1,895 $\pm$ 225	856 $\pm$ 205	101 $\pm$ 4
Fungal cytoplasmic supernatant	100.0 μg ml ⁻¹	1,025 $\pm$ 125	721	95
	500.0 μg ml ⁻¹	1,434 $\pm$ 194	777	93
<i>Ineffective biotic elicitors</i>				
Fungal culture medium control†	5.0 μg ml ⁻¹	<20	132	97
Soybean cell wall†	200.0 μg ml ⁻¹	<20	115	105
Soybean cytoplasmic supernatant†	200.0 μg ml ⁻¹	<20	142	98

Amounts of glyceollin accumulated and degraded were measured at 24 and 8 h, respectively, and rates of synthesis at 18 h after incubation of cotyledons with elicitors, as described in Fig. 1. For preparation of fungal extracellular metabolite, *Phytophthora megasperma* var. *sojae* was cultured as described in Fig. 1. After fungal mycelium was removed by filtration, the culture fluids were lyophilised, dissolved in 10 mM Tris-HCl (pH 7.2), and passed through a column of Sephadex G-50 equilibrated with the same buffer. Fractions eluted in the column void volume were used. Fungal cytoplasmic supernatant was prepared by centrifuging a mycelial homogenate of the same fungus in 10 mM Tris-HCl (pH 7.2) at 80,000 g for 30 min and by dialysing the resulting supernatant against the same buffer. Fungal culture medium control was the fraction corresponding to the fungal extracellular metabolite fraction but the medium was not inoculated with the fungus. Soybean cell wall and cytoplasmic supernatant were prepared using the same methods described for preparation from the fungus. Means and standard errors are indicated where 3 to 5 replicate experiments were run, and other values are means of two replicate experiments.

* Actual values of incorporation of ¹⁴C-phenylalanine into glyceollin (c.p.m.) or of amounts of glyceollin degraded (μg) in the control cotyledons.

† Abiotic chemicals or biotic substances which did not induce glyceollin accumulation at all concentrations tested (0.033–20 mM for abiotic chemicals and 5–1,000 μg ml⁻¹ for biotic substances). Concentrations of effective abiotic elicitors were those approximately optimum in inducing glyceollin accumulation. Concentrations of all biotic substances were based on glucose equivalents measured by anthrone assay.

tinguished from the specific response associated with disease resistance, as it seems that the fungal metabolites operate differently from the abiotic elicitors.

The results presented here suggest that it may be possible to discover a new type of plant protectant, protecting against infection not through intrinsic antibiotic activity, but through an effect on plant-pathogen interaction resulting in higher accumulation of phytoalexins.

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The blind octopus, *Cirrothauma*

THE rare deep-sea octopod *Cirrothauma murrayi* Chun 1910 was first described from a single specimen caught during the Michael Sars Expedition of 1910 (ref. 1). Until now it has been caught only four more times². We describe here three specimens of this species that were recently caught during biological cruises of the RRS Discovery (Fig. 1). All of these animals, including the Discovery ones, have been caught at depths of more than 1,500 m, except one that was dip-netted through the ice of the Arctic Ocean³.

Cirrothauma belongs to a poorly known family, the Cirroteuthidae, which have cirri along the arms, a pair of fins, a deep interbranchial web and no radula¹. *Cirrothauma* is reddish-brown semi-transparent and gelatinous in consistency (Fig. 1). Photographs of other cirroteuthids taken with deep-sea cameras have shown them swimming close to the bottom and looking like medusae, with head down and arms and web widely extended^{4,5}. In other photographs they are swimming backwards with a more typical cephalopod posture⁵.

The eyes are remarkable, being simple open cups, with no trace of lens, iris or ciliary body (Fig. 2). The cup is closed by a transparent continuation of the sclera and covered by a thin gelatinous layer less than 1 mm thick. The eyes were only 3 mm in diameter in Chun's animal, the mantle length being 40 mm. Our specimens are larger (155 mm (A), 130 mm (B) and 220 mm (C) mantle length) with an oval pupil 9 mm $\times$ 12 mm in the smallest (Fig. 2). The retinæ are better developed than is implied from Chun's description¹ (Fig. 3a). However, the rhabdoms are not regularly packed as they are in *Octopus*, and there is no limiting membrane covering their outer ends, which therefore turn and taper irregularly. In one of the Discovery specimens part of the retina was folded, to make a series of chambers, lined by separate, single rhabdoms, looking, as Chun put it, "like little flames"¹ (Fig. 3b). The retina of specimen B therefore seems to be in a degenerating condition similar to that of some deep-sea fishes⁶.

The optic nerves are very long and pass through a large mass of tissue, the 'white body', to a minute optic lobe (Fig. 2). The structure of this is very simple, it lacks the peripheral layers of small cells that are characteristic of cephalopods with good vision. The vertical lobe and other parts of the visual memory system, so well-developed in *Octopus vulgaris*⁷, are reduced. It is difficult to understand what use may be made by the animal of a photoreceptor system such as this, with neither lens nor pin-hole aperture. That the animal has some use for photoreceptors is shown by the fact that the epistellar bodies, photosensitive vesicles near the stellate ganglion, are especially large in *Cirrothauma*.

There are large sac-like statocysts, suspended by muscles at some distance from the brain. As in other octopods there are inner and outer sacs separated by a perilymph (Fig. 4). The maculae are long oval patches with small circular statoliths not covering the whole area. There are no maculae neglectae. The

Fig. 1 *Cirrothauma murrayi* Chun 1910 photographed on board RRS Discovery while still just alive. The posterior end of the mantle is translucent and parts of the fins are transparent. Marks from the nets can be seen on the skin. One eye is visible at the side of the long, slender funnel. The arms are united by the web along most of their length. The stalked 'suckers' lie towards the tips of the arms. Specimen B caught at 3,000–3,500 m, mantle length 130 mm, funnel length 61 mm.

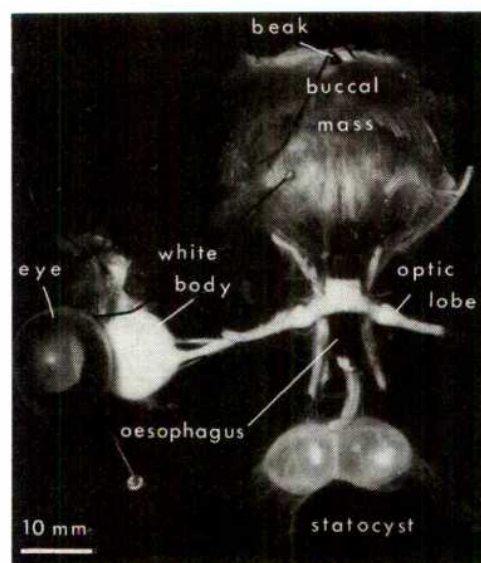
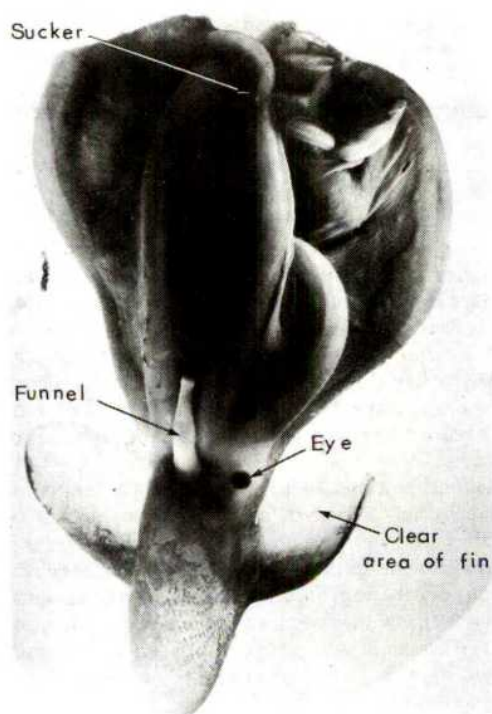


Fig. 2 The central nervous system, one eye, the statocysts and the buccal mass after dissection. The black rostral tip of the lower beak projects beyond the lips. The oesophagus is heavily pigmented and can be seen just behind the brain. Specimen B.

crista runs round the circumference of the sac, constricting it somewhat by a waist to form lateral and medial parts. There is a single anticrista, as in other octopods. The large volume of endolymph is presumably related to the need for the crista to detect slow angular rotations. This condition resembles that found in *Vampyroteuthis*, but the latter has several anticristae, as in decapods⁸.

The cirri are characteristic of the Cirroteuthidae and are absent from all other cephalopods with the exception of *Vampyroteuthis*. *Cirrothauma* has a longitudinal row of cirri on either side of the single row of suckers. Though not shown in Fig. 1, they can probably extend to a considerable length and are presumably tactile organs. They were stretched outwards and away from the arms in the cirroteuthids photographed in the sea⁵. Sections of the Discovery specimens show that the tactile centres of the brain are well-developed, unlike the visual system, and perhaps the cirri provide the main source of information about the surrounding conditions and for the detection of food. There are 38 suckers along each arm of our specimen of 155 mm mantle length (A) and 36 on the Chun (or 'type') specimen¹. Chun recognised two distinct sets of suckers, one being unique amongst cephalopods¹. He described the latter as 'tiny suckers situated on long, plump, spindle-shaped gelatinous stalks... 4–5 mm long in the middle of the arms and gradually become shorter towards the tips and proximally'. He suggested that they had lost their function as there was no opening. That they are suckers, even though they do not have an orifice, is shown in histological sections by the presence of a cuticle very similar to that found covering the infundibular surface of the suckers of *Octopus vulgaris* and other octopods⁹. In view of the presence of this sucker cuticle, it seems unlikely that Chun's¹ reserved interpretation that there were 'certain similarities with luminous organs' can now be upheld. Dr P. J. Herring (Institute of Oceanic Sciences) has also looked at the sections for us and can find no trace of light organs. The second type of sucker does have an opening and these suckers lie near the mouth, being the first six in both Chun's animal and in specimen A. The opening of the sucker is restricted and does not lead to a suction chamber such as is usually found in octopods⁹. There is a very shallow cup with radial muscles and a typical cuticle which, like that in other octopods, can be shed as a disk⁹.

The buccal mass is large as is the rostrum of the lower beak, which projects beyond the lips (Fig. 2). As in other cirroteuthids the radula is absent^{1,10}, in its place there is a fleshy tongue-like

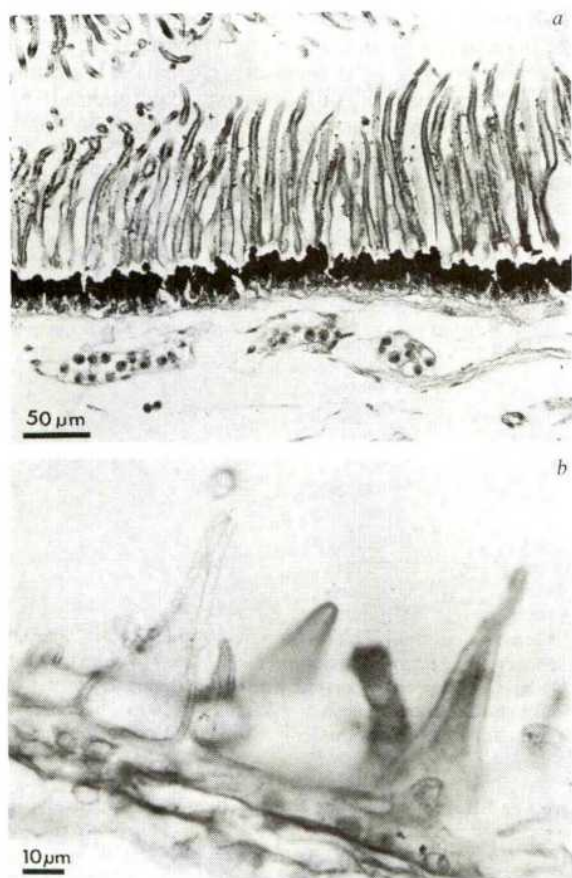
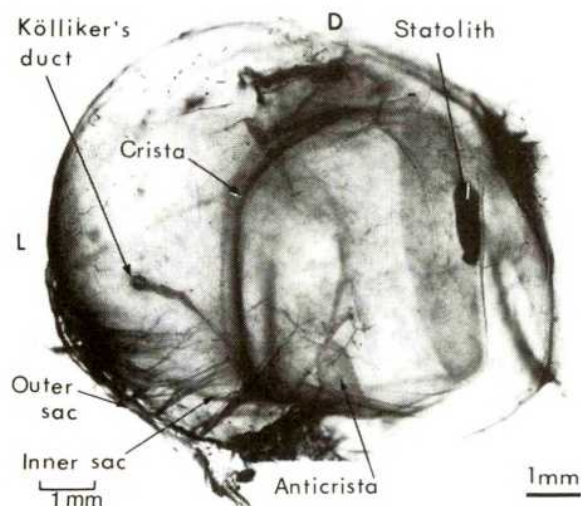


Fig. 3 *a*, Section of the retina of specimen C. *b*, Isolated rhabdoms from a sac formed by folding of the retina of specimen B.

structure attached ventrally along most of its length. At the front of this 'tongue' there is a large papilla, perhaps the salivary papilla.

The funnel is extremely long and slender¹, being 47, 61 and 185 mm in animals A, B and C, respectively (Fig. 1). The mantle opening is small and closely surrounds the base of the funnel. The function of this long funnel is not known but it could be important in directing fine jets of water during delicate manoeuvring movements while swimming upside down in the

Fig. 4 Left statocyst from specimen A seen from in front. The statolith is displaced and seen edge on near to the macula. D, dorsal; L, lateral.



medusoid form. Perhaps its jets of water are used to stir up the sediment on the seabed to expose small prey, or possibly to bury the eggs in soft sediment.

The cirroteuthids differ from all other octopods and resemble decapods in the presence of fins (Fig. 1). These, like the cirri, recall the condition in *Vampyroteuthis*¹¹. It seems probable that these deep-sea animals still retain characteristics common to both octopods and decapods. In this connection it is interesting that *Cirrothauma* and another cirroteuthid of which we also have sections, both have a system of giant nerve fibres¹¹, which are usually only associated with decapods¹². We hope that further study of the Discovery specimens may help to settle several problems of structure, function and mode of life of these curious animals.

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Spike frequency adaptation in amphibian sensory fibres is probably due to slow K channels

SPIKE frequency adaptation (SFA)—a decrease in firing frequency during the action of a maintained stimulus—is a mechanism for the input-output transformation of a signal and it is common at many levels of the animal kingdom. The occurrence of SFA in amphibian sensory nerves¹⁻⁴ offers an opportunity to explain the phenomenon at the membrane level in terms of the Hodgkin-Huxley (HH) theory⁵. Here we show that SFA in amphibian nerve is probably due to a current through slow K channels, and we suggest that these channels must also be responsible for different cases of SFA in nerve cells of various types.

The HH model for the node^{1,6} gives, for constant current steps, rhythmic firing but not SFA⁷. Further mechanisms with relatively long time constants are obviously needed to account for SFA. As SFA lasts only from several ms to a few tens of ms it is evident that slow Na (ref. 8) and fast K (ref. 9) inactivations, with time constants of several hundred ms and even 1 s, cannot be a source for SFA. Electrogenic pumping is also excluded (for refs and discussion see ref. 10). K⁺ accumulation near the node¹¹ can be expected, by analogy with squid axon¹², not only to fail to cause SFA but also to give inappropriate changes of successive overshoot and undershoot amplitudes in a train, decreases rather than increases. Therefore, we consider that the necessary mechanism involves the currents flowing through the slow K channels. The addition of slow K channels with the properties we have measured gives calculated SFA with most of the properties measured by others. Also, there are indications of slow K channels in amphibia in which SFA is usually observed, *Xenopus*

laevis^{2,4,13}, *Rana esculenta*^{3,4,14} and *R. ridibunda*^{4,15,16}. In the case of *R. ridibunda* many characteristics of the channels have been determined¹⁶, and they are used here for SFA calculations. We modified the HH model to present the overall K current (I_K) of the node as the sum of two components: a fast (I_{K1}) and a slow (I_{K2}) component.

The fast channel in *R. ridibunda* seems to behave like the familiar K channel in *R. pipiens*^{1,17}. The two channels were attributed to sensory nerve on the basis of their corresponding $n_{1\infty} - E$ and $n_{2\infty} - E$ curves on the E (membrane potential) axis, as had been done for *R. esculenta*¹⁸. I_{K1} can thus be presented by the HH-Dodge model for *R. pipiens*¹. I_{K2} can also be presented by an HH-type model, but differing in behaviour with E of the HH variable n_{II} describing the E -dependent gating kinetics of the slow K channel (ref. 16 and Fig. 1):

$$I_{K2} = g_{K2}(E - E_{K2}) \quad (1)$$

$$g_{K2} = \bar{g}_{K2} n_{II}^4 \quad (2)$$

where g_{K2} , $\bar{g}_{K2}$ are, respectively, the slow K channel conductance and its maximum value; E_{K2} is the K equilibrium potential for I_{K2} .

In *R. ridibunda* the ratio $\bar{g}_{K2}/\bar{g}_{K1}$ is usually 0.2–0.8, depending on the frog specimen¹⁶. E_{K2} is unknown. It was taken to be equal to E_{K1} , that is, E_K in *R. pipiens* (–75 mV). The variable n_{II} can be represented fully by two functions: the steady-state relationship $n_{II\infty} - E$ and its kinetic characteristic $\tau_{nII} - E$. Both are given in Fig. 1.

For computations of spike trains, Hille's version of the HH-Dodge model¹⁷ was used. Small variations of maximum Na conductance with E (the term $(1 - E/183)$) were neglected so that we could follow quantitatively the critical firing level in terms of the theoretical relationship between firing potential and parameters entering the HH model¹⁹. To induce rhythmic activity the $h_{\infty} - E$ curve was shifted by 15 mV in the depolarising direction. Computations were carried out on a BESM-6 computer (Russian index) with a Fortran program.

Figure 2 shows the computed firing behaviour for the case when the contribution of I_{K2} into the overall I_K was varied by variation of $\bar{g}_{K2}$, that is, the density of the slow K channels. At $\bar{g}_{K2} = 0$ the firing is purely rhythmic (Fig. 2a). However, SFA is well expressed even with $\bar{g}_{K2}$ as small as 10% of $\bar{g}_{K1}$ (Fig. 2b), and quickly increases with $\bar{g}_{K2}$ (Fig. 2c,d) until damping of repetitive responses is complete (Fig. 2e).

Although Fig. 2 shows the simplest case, when an increase in I_{K2} (a–e) is due to $\bar{g}_{K2}$, it probably qualitatively covers most of the previously described basic features of SFA in amphibian nerve^{1–4}. One of them is a progressive rise in time of the overshoot and undershoot (for spikes following the first) amplitudes with nearly constant firing level. This is clearly seen in Fig. 2c and d. Further, the spike trains in amphibian nerves are finite, and this can be seen in Fig. 2d where, with sufficient density of slow K channels (that is, with sufficiently large $\bar{g}_{K2}$), only one spike can be produced (Fig. 2e), which may be considered an extreme case of adaptation.

Thus, even the simplest model considered can account not only for SFA as such (a decrease in firing frequency with time) but also for accompanying phenomena (the character of changes of the overshoot and undershoot amplitudes and finite spike trains). Also any variation of parameters in equations (1) and (2) leading to greater I_{K2} will lead to more pronounced SFA. This provides a variety of conditions for critical damping, as is the case in actual experiments (compare records of spike trains in refs 1–4). A proper choice of the starting value h_0 (refs 4, 7) and consideration of Na^+ accumulation near the inner surface of the nodal membrane¹⁰ and K^+ accumulation near its outer surface¹¹, would make the agreement between theory and experiment even better. There is, however, little hope of obtaining full details of SFA for a particular node, because this requires knowledge of the HH picture of all the currents involved and of the accompanying processes (such as Na^+ and K^+ accumulation, see above) on the same node. We aimed to show that the slow K channels are responsible for SFA in the node, and that this SFA

can be modified further by the various factors discussed here. The construction of models seems to be the best way to study basic features of SFA, so as to adjust relevant HH parameters and those of accompanying processes to fit experimental SFA or to look for interesting phenomena which can then be sought in an experiment. Our results indicate that slow K channels are responsible for SFA in amphibian nerve. In other amphibian

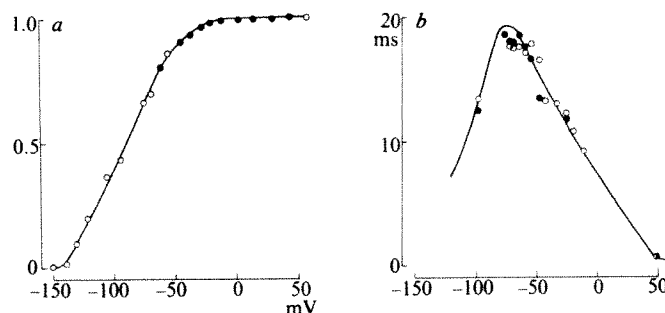


Fig. 1 a, Function $n_{II\infty}(E)$ for slow K channel used to compute the sensory nerve firing behaviour in Fig. 2. $\circ$, $n_{II\infty}$ values obtained by fitting $I_K - t$ curves associated with depolarising E steps from various values of E_1 (given by abscissa) to $E_2 = 50$ mV by searching with least squares the parameters of the equation for overall I_K :

$$I_K(t) = g_{K1}(t)(E - E_{K1}) + g_{K2}(t)(E - E_{K2})$$

where I and II refer to fast and slow K channels, respectively. I_{K2} was measured in 128 mM KCl and 3×10^{-4} mM tetrodotoxin, with leakage current subtracted. As $E_{K1} = E_{K2}$, $n_{II\infty}(50 \text{ mV}) = n_{I\infty}(50 \text{ mV}) = 1$, and there is no K^+ accumulation near the node in this solution¹⁶, the equation acquires the HH form:

$$g_K(t) = \bar{g}_{K1} \cdot \left[1 - (1 - n_{I\infty}(E_1)) \exp\left(-\frac{t}{\tau_{nI}}\right) \right]^{x_1}$$

$$\bar{g}_{K2} \cdot \left[1 - (1 - n_{II\infty}(E_1)) \exp\left(-\frac{t}{\tau_{nII}}\right) \right]^{x_{II}}$$

The terms: $\bar{g}_{K1}$, $n_{I\infty}(E_1)$, τ_{nI} , x_1 , $\bar{g}_{K2}$ (where $\bar{g}_{K2} = (1 - \bar{g}_{K1})$), $n_{II\infty}(E_1)$, τ_{nII} , x_{II} in the latter equation were given values so that they fitted the whole family of experimental $I_K - t$ curves (now assumed to be the shape of the $g_K - t$ curves) associated with a set of E_1 . Parameters for channel I happened to be the same as for the K channel in *R. pipiens*^{1,17}. x_{II} was found to be 4 (as x_1), $n_{II\infty}(E_1)$ ($\circ$), τ_{nII} ($\bullet$) in b. Details of the fitting procedure are given elsewhere¹⁶. $\bullet$, $n_{II\infty}$ values obtained from the relationship $g_{K2\infty} - E$ varying as $(\bar{g}_{K2\infty}/\bar{g}_{K1})^{1/4}$. The relationship $g_{K2\infty} - E$ was constructed from amplitudes of instantaneous I_K associated with repolarising E steps from various values of E_1 (given by abscissa) to the same value of E_2 , –100 mV (details are given in refs 15, 16). The $n_{II\infty} - E$ curve was drawn by eye and its ordinates were fed into the computer in 5-mV steps along the abscissa. b, Function $\tau_{nII}(E)$ for the slow K channel used for computation of sensory nerve firing behaviour in Fig. 2. τ_{nII} values were calculated from experimental time constants τ_{K2} determining the decline of g_{K2} tails following repolarisation from constant value of E_1 , 67 mV, to various values of E_2 (given by abscissa) by the following equation:

$$\bar{g}_{K2} \cdot \left[(n_{II\infty}(E_1) - n_{II\infty}(E_2)) \exp\left(-\frac{t}{\tau_{nII}}\right) + n_{II\infty}(E_2) \right]^4$$

$$= (g_{K20} - g_{K2\infty}) \exp\left(-\frac{t}{\tau_{K2}}\right) + g_{K2\infty}$$

where g_{K20} is the g_{K2} value at the moment just after a repolarising step, and $g_{K2\infty}$ is its steady-state value at E_2 . The solution was obtained for $t = \tau_{K2}$ assuming $n_{II\infty}(67 \text{ mV}) = 1$ (see a), $g_{K20} = \bar{g}_{K2} \cdot n_{II\infty}^4(67 \text{ mV}) = \bar{g}_{K2}$, $g_{K2\infty} = \bar{g}_{K2} \cdot n_{II\infty}^4(E_2)$. For $n_{II\infty}$ of ~0.3 (that is, for $E_2 \geq -110$ mV), $\tau_{nII} \approx \tau_{K2}/n_{II\infty}(E_2)$. τ_{nII} were scaled to 22°C (as in Hille's equations for τ_n in *R. pipiens*¹⁷) with a Q_{10} of 3.0. $\circ$, τ_{nII} measured, as described, with E_1 40 mV; $\bullet$, the same, with E_1 500 mV; $\bullet$, τ_{nII} obtained from depolarising E steps as described in a. The smooth curve was drawn by eye and its ordinates were fed into the computer in 5-mV steps along the abscissa.

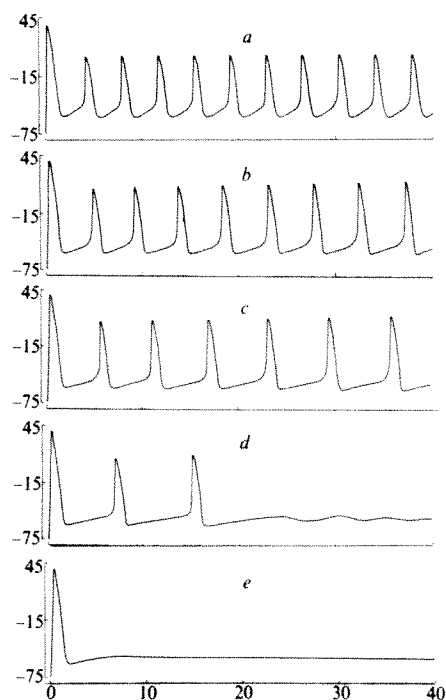


Fig. 2. Effect of the slow K current $I_{K_{II}}$ on firing behaviour of amphibian sensory nerve associated with a constant current step of 0.9 nA. Increase in $I_{K_{II}}$ is due to increase in $\bar{g}_{K_{II}}$ (0, 10, 20, 30 and 40%, respectively, in a, b, c, d and e of $\bar{g}_{K_{II}}$ (maximum K conductance through the fast K channels)). As $I_{K_{II}}$ in *R. ridibunda* is an additional process to the *R. pipiens* sensory nerve¹⁶, all the basic data necessary for this computation were those tabulated for *R. pipiens* by Hille¹⁷, except for h_0 which was chosen to be 0.9286 to induce the repetitive activity as in a. Abscissa: time (ms), ordinate: membrane potential (mV).

sensory nerves capable of SFA, the slow K channels are likely to be generally similar to those in *R. ridibunda*¹³, so that the physiological basis for SFA must be common to different amphibians.

Slow K channels might also provide a mechanism additional to or underlying that described¹⁸ for sensory compared with motor fibres. Sensory fibres respond to a constant current step by producing a train with increasing interspike intervals, whereas motor fibres respond by producing one or very few spikes³. Thus, motor fibres can be considered quickly adapting. The $n_{\infty}-E$ curve for motor fibres was found to be displaced from that for sensory fibres in a hyperpolarising direction¹⁸, which damps repetitiveness (our unpublished calculations). However, this displacement and consequently the mechanism for sensory compared with motor fibres may be the result of greater $\bar{g}_{K_{II}}$ in motor than sensory fibres, for $\bar{g}_{K_{II}}$ is activated by more negative ranges of membrane potential than is $\bar{g}_{K_I}$ (refs 15, 16). Thus, in Fig. 1 the 0.5 n_{∞} value is at $E = -92$ mV whereas the 0.5 n_{∞} value for *R. ridibunda* usually lies between -50 and -60 mV¹⁶, as 0.5 n_{∞} for *R. pipiens*^{1,17}. Then the shift of the curve of $n_{\infty}-E$ for overall $\bar{g}_K$ (as measured before¹⁸) will increase with $\bar{g}_{K_{II}}$, that is, with the density of the slow K channels. It is tempting to suggest that neurones in the ventral roots synthesise more slow K channels than neurones in the dorsal roots.

The sensory nerves of *R. pipiens* usually seem to have only fast K channels. This follows from I_K measurements, indicating that they obey¹ the Cole-Moore translation principle²⁰, and from noise measurements suggesting a two-state open-closed transition for the K channels involved²¹. Both tests would fail if I_K in this node were due to more than one population of K channels (it was the analysis of I_K currents associated with the Cole-Moore translation test that led to the discovery of the slow K channels in *R. ridibunda*^{15,16}). However, as SFA also occurs (rarely) in *R.*

*pipiens*¹, our hypothesis suggests that a slow K channel must occur, if rarely, in this specimen as well, but that the yield of the gene product for the slow K channel is lower here than in other species.

The only other example for which SFA has been explained in terms of the HH theory concerns neurones from the mollusc *Archidoris*²². The SFA phenomenology here differs considerably from that in amphibian nerves, especially in that spike trains here are finite²². However, this is the picture that results from computation of the firing behaviour when the current through the slow K channel involved (which considerably differs from that in the node) is taken into account. Thus, in two markedly different cases of SFA (in the node and the neurone), the current through the corresponding slow K channel is sufficient to describe basic characteristic features of SFA. There are many different types of slow K channels in biological membranes (reviewed in ref. 23) and, except for (or together with) the cases where an electrogenic pump is involved²⁴, the diversity in SFA phenomenology in nerve cells of different types is probably mainly due to diversity of slow K channels. The mechanism discussed here might also apply to adaptational behaviour of sustained and transient receptors responding to a stationary component of generator potential, as shown in Fig. 2b, c and d, e, respectively.

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Labelling of benzodiazepine receptors *in vivo*

THE presence of benzodiazepine receptors has been demonstrated using isolated membrane preparations from the mammalian central nervous system¹⁻³. Specific binding of ³H-diazepam to synaptosomal membranes is maximal at temperatures of 0-4 °C, but reduced by more than 95% at physiological temperatures⁴. Although the inhibition of ³H-diazepam binding by other benzodiazepines *in vitro* closely parallels their pharmacological potencies *in vivo*, failure to demonstrate specific binding in physiological conditions raises questions as to whether receptor binding occurs *in vivo*. We report here that benzodiazepine receptors can be demonstrated in the intact

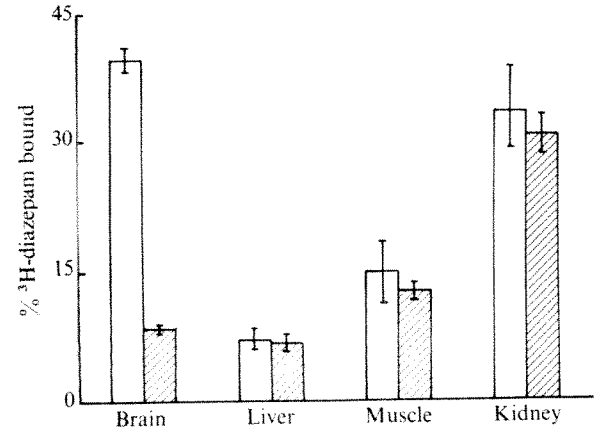
animal following intravenous administration of ³H-diazepam. The labelling of benzodiazepine receptors *in vivo* permits studies of receptor occupancy in various pharmacological and physiological conditions as well as autoradiographic localisation of the receptor *in situ*.

Adult male Sprague-Dawley rats (125–175 g) were used in all studies. ³H-Diazepam (39.08 Ci mmol⁻¹, NEN) was diluted with 50% ethanol to a final volume of 300 μl and administered through the lateral tail vein. Studies in this (unpublished) and other laboratories⁵ have demonstrated that the maximum brain concentration of diazepam occurs within 1–2 min of intravenous administration. At this time 95% of the radioactivity in brain is authentic diazepam, as demonstrated by thin layer chromatography⁵. Animals were decapitated 1 min after injection and the tissues to be assayed were immediately dissected, weighed and frozen on dry ice (–70 °C). Studies designed to test the effects of freezing on the amount of specifically bound ³H-diazepam revealed no differences between fresh and frozen samples. Therefore, subsequent studies were carried out using frozen specimens, giving greater efficiency and control over assay and homogenisation time.

For assay of membrane-bound ³H-diazepam, tissues were homogenised in 25 volumes of ice-cold 50 mM Tris-HCl buffer (pH 7.4) using a Brinkmann polytron (20 s, full speed). Aliquots of the homogenate were sampled for total and membrane-bound ³H-diazepam. The latter was determined by filtering the homogenate (0.1–1.0 ml) through Whatman glass fibre filters (GF/B) followed by two rinses of ice-cold Tris-HCl buffer (5 ml each). The filters were then placed in scintillation vials containing 10 ml of Aquasol (NEN), shaken thoroughly and counted with a Beckman LS-250 liquid scintillation counter.

Membrane-bound radioactivity was linear with tissue concentration in homogenates containing up to 20 mg of brain tissue (wet weight). Preliminary studies were carried out to determine whether the specific membrane-bound radioactivity was a result of *in vitro* binding that might have occurred artefactually following homogenisation. In an experiment comparing the amount of specifically bound ³H-diazepam with post-homogenisation incubation time, both specific and nonspecific binding were maximal at the earliest time tested (30 s post-homogenisation) and did not change over a 60-min period. As saturation of benzodiazepine receptors *in vitro* occurs approximately 10 min after incubation^{4,6}, these data support the assumption that the binding observed following intravenous administration had occurred *in vivo*. The results also demonstrate the stability of the drug-receptor complex when the preparation is kept at 0–4 °C.

Fig. 1 Stereospecific inhibition of ³H-diazepam binding *in vivo*. Rats were injected with 50 μCi ³H-diazepam in combination with the enantiomers of B9 or B10 (700 μg kg⁻¹). Values represent $\bar{X} \pm \text{s.e.m.}$ in brain ($n = 8$), liver ($n = 6$) and other tissues ($n = 2$). Per cent of ³H-diazepam bound is the amount of ³H-diazepam in the homogenate retained by the 6F/B filter divided by the amount of ³H-diazepam in an equal volume of homogenate $\times 100$. ■, Active isomers (B9(+), B10(+)); □, inactive isomers (B9(–), B10(–)).



Co-injection of the ³H-diazepam with unlabelled clonazepam (250 μg kg⁻¹) or the pharmacologically active benzodiazepine enantiomers B9(+) and B10(+) (700 μg kg⁻¹) dramatically reduced membrane-bound ³H-diazepam (40.7 ± 2.1% ($n = 5$) compared with 7.72 ± 1.54% ($n = 5$) bound) whereas the total concentration of ³H-diazepam in brain was unaffected. Substitution of the pharmacologically inactive enantiomers B9(–) or B10(–) did not produce any significant decrease of membrane-bound ³H-diazepam (39.8 ± 1.2% bound). Homogenisation of the tissue in buffer (50 mM Tris-HCl, pH 7.4) containing 3 μM unlabelled diazepam resulted in a rapid displacement of more than 90% of membrane-bound radioactivity. The latter experiment agrees well with observations *in vitro* indicating that more than 90% of the ³H-diazepam bound to crude synaptosomal fractions of brain is displaced by this concentration of diazepam^{1,3}.

Table 1 Regional distribution of benzodiazepine receptors *in vivo*

Region	Specific ³ H-diazepam binding (pmol per g tissue)	Nonspecific binding (% of total binding)
Cortex	12 ± 2.0	12
Hypothalamus	8.7 ± 0.7	19
Midbrain	7.6 ± 0.7	20
Striatum	6.8 ± 1.7	32
Septal region	6.2 ± 0.2	17
Cerebellum	6.2 ± 0.7	50
Pons medulla	5.5 ± 0.6	20

Rats were injected intravenously with 50 μCi ³H-diazepam. The various brain regions were hemisected, one half of each region being homogenised in 50 mM Tris buffer (pH 7.4) with or without 3 μM diazepam, and assayed in triplicate within 15 min of homogenisation. Specific binding is defined as the amount of ³H-diazepam bound after homogenisation in Tris buffer minus the amount bound after homogenisation with 3 μM diazepam. Values represent the mean ± s.e.m. of brain regions from three rats.

As *in vitro* studies have shown that benzodiazepine receptors are highly localised to brain^{1,4}, it was of interest to study the tissue distribution of ³H-diazepam binding *in vivo*. Figure 1 shows the binding of ³H-diazepam in brain and peripheral tissues with and without co-injection of a pharmacologically active benzodiazepine. Although ³H-diazepam binds to membranes of all the peripheral tissues studied, only the binding in brain is displaced in a stereospecific fashion. Similar results have been reported *in vitro*⁴. Finally, we investigated the regional distribution of benzodiazepine receptors in brain (Table 1). The rank order concentration of benzodiazepine receptors in various cortical and subcortical areas is identical to that observed in the *in vitro* assay⁴.

Our results demonstrate the feasibility of labelling benzodiazepine receptors *in vivo* in physiological conditions. These receptors are highly localised to the CNS, and to cortical areas in particular. Displacement of specific binding occurs only with pharmacologically active benzodiazepines, thus supporting a role for these sites as pharmacological receptors mediating the action(s) of these drugs within the CNS.

In addition to establishing specific binding of ³H-diazepam to benzodiazepine receptors in physiological conditions, *in vivo* labelling of benzodiazepine receptors may also permit further studies on receptor changes during various pharmacological and/or physiological states, as well as autoradiographic visualisation of the receptor. Similar *in vivo* studies using ³H-flunitrazepam have also been carried out (R. Chang and S. Snyder, personal communication).

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An endogenous protein modulates the affinity of GABA and benzodiazepine receptors in rat brain

BIOCHEMICAL^{1,2} and neurophysiological³⁻⁵ evidence has suggested that benzodiazepines may relieve anxiety facilitating the synaptic action of γ -aminobutyric acid (GABA), an important neurotransmitter in the brain of mammals, including man. As benzodiazepines fail to increase the turnover of GABA stored presynaptically as would be expected if they were to act as indirect GABA agonists, and since it has been difficult to demonstrate a direct agonistic action of the benzodiazepines on GABA postsynaptic receptors²⁻⁷ *in vitro*, the molecular mechanism whereby benzodiazepines facilitate GABAergic transmission is still unknown. Independent investigators⁸⁻¹⁰ have recently reported the presence of a high affinity, saturable, stereospecific binding site for benzodiazepines in synaptic membrane preparations obtained from brain of different animal species, including man. This high affinity binding is now used to determine the therapeutic potency and to study the mode of action of benzodiazepines in anxiety.

Any known transmitter, including GABA, fails to compete with the benzodiazepines for their specific brain receptors; however, the brain distribution pattern of these benzodiazepine receptors is similar to that of the Na^+ -independent high affinity binding sites for GABA (8,9). This and analogies in the pharmacological profile of GABA-mimetics and benzodiazepines^{2,11} has suggested to us that studies of the mechanism whereby benzodiazepine receptors interact with the Na^+ -independent GABA receptor sites may help to understand the mode of action of these antianxiety drugs.

The discovery that benzodiazepine receptors are present in the central nervous system has also led to the proposal¹² that: "by analogy with the discovery of opiate receptor, the benzodiazepine receptor assay may even offer a simple means for the discovery of a naturally occurring ligand for such receptor: the brain's own antianxiety substance".

Recently, we reported that a small thermostable protein ('endogenous inhibitor') which noncompetitively reduces the affinity of GABA for its Na^+ -independent recognition site, could be extracted from brain preparations enriched with synaptic membranes¹³. This endogenous inhibitor is thermostable (at 95°C for 10 min), has acidic characteristics, and when

purified 1,500-fold by gel filtration, ion-exchange chromatography, and preparative polyacrylamide gel electrophoresis, gives on 10, 15 and 20% SDS polyacrylamide gel only one major protein band equivalent to a molecular weight of ~15,000. The present report shows that pharmacologically-active benzodiazepines modulate the affinity of GABA for its recognition

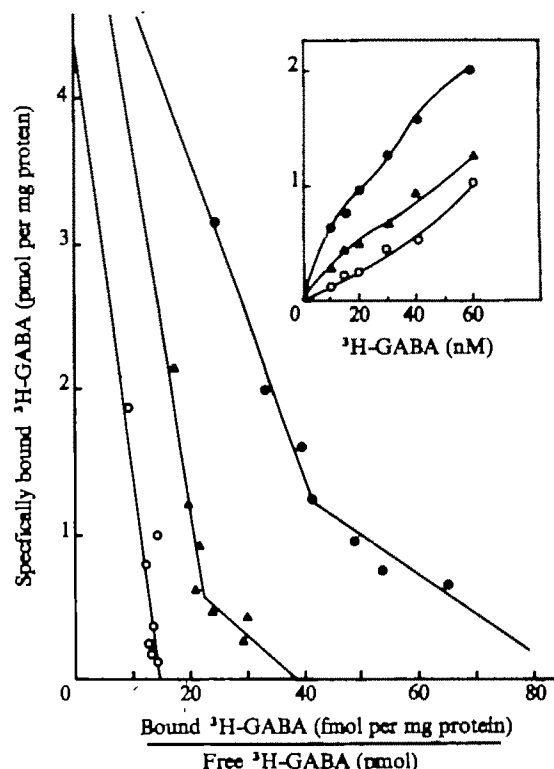


Fig. 1 Scatchard analysis of interaction between diazepam and 'endogenous inhibitor' of Na^+ -independent ^3H -GABA binding. Crude synaptic membranes from rat cerebral cortex¹⁵ were frozen at -20°C for 24 h and treated for 30 min at 37°C with 0.01% Triton X-100. After centrifugation at 40,000g for 10 min the pellet was dispersed with a polytron in 50 volumes of 50 mM Tris-citrate (pH 7.1) without Triton X-100. This operation was performed twice. Specific GABA binding was calculated by subtracting from the binding which occurred by incubating the membranes at 4°C for 5 min with ^3H -GABA, the binding that occurred by incubating with ^3H -GABA plus 10^{-3}M unlabelled GABA. Only ^3H -GABA (40 Ci mmol $^{-1}$) was used when the lowest concentrations of ligand (1-30 nM) were tested, and a constant amount of ^3H -GABA (30 nM) plus various concentrations (30-120 nM) of nonradioactive GABA when the highest concentrations of the ligand were tested. The ligand tested was contained in 100 μl which was incubated with a standard aliquot (200 μg protein) of membranes dispersed in 900 μl of 50 mM Tris-citrate (pH 7.1). After incubation the reaction was terminated by centrifuging at 48,000g for 10 min. The supernatant was discarded and the pellet rapidly rinsed with cold H_2O and suspended in NCS (Amersham-Searle solubiliser) before counting. To study the action of the endogenous inhibitor, membranes were incubated at 0°C for 15 min, with 7.5 μg of 500-fold purified endogenous inhibitor before the addition of ^3H -GABA. When the effect of diazepam was studied membranes were preincubated at 0°C for 15 min with $8 \times 10^{-7}\text{M}$ diazepam before addition of inhibitor. Membranes incubated at 0°C without the inhibitor or diazepam served as controls. Scatchard analysis revealed two affinity components for GABA binding. The apparent K_D for the low affinity component was: 111; 240 and 286 nM for control, diazepam + endogenous inhibitor and endogenous inhibitor, respectively. The apparent K_D for the high affinity component was: 28 and 33 nM for control and diazepam + endogenous inhibitor, respectively. A high affinity component for ^3H -GABA binding was not measurable in membranes preincubated with the endogenous inhibitor alone. In the insert the values of the specifically bound (SB) ^3H -GABA are plotted against the concentrations of ^3H -GABA in the incubation mixture. Proteins were determined with the method of Lowry¹⁸. ●, Control; △, diazepam + inhibitor; ○, inhibitor only.

site by competing with the endogenous inhibitor. In addition, this endogenous protein reduces the apparent affinity of diazepam for its recognition site. To study whether diazepam could change the activity of the endogenous inhibitor, the inhibitor was purified 500-fold using a previously described procedure¹³. In brief, the entire rat brain was homogenised in 5 mM Tris-citrate buffer (pH 7.1). After centrifugation, the supernatant was incubated at 95°C for 10 min and successively dialysed filtered on CF 50A Amicon membrane and chromatographed on Sephadex G-100 column. It was then purified further with Dowex 50X8 ion-exchange chromatography where it behaved as an acidic protein.

As shown in Fig. 1a, the addition of this purified inhibitor to a crude preparation of synaptic membranes free of endogenous inhibitor (the inhibitor was removed by freezing and thawing and by treatment with Triton X-100, for details see Fig. 1 legend), resulted in a substantial change of the kinetic characteristics of Na⁺-independent ³H-GABA binding. Scatchard plot analysis of these data indicates that the addition of purified endogenous inhibitor to crude synaptic membrane preparations concealed the Na⁺-independent high affinity recognition sites for GABA and also changed the kinetic characteristics of the low affinity GABA binding sites (see Fig. 1). If the membranes were preincubated with diazepam, the action on the endogenous inhibitor on both high and low affinity components of GABA binding was reduced (Fig. 1). The data of Fig. 1 indicate that the action of the endogenous inhibitor on GABA binding is apparently noncompetitive, and suggest a negative cooperative interaction between the inhibitor and the GABA recognition sites. Diazepam added in concentrations up to 10⁻⁵ M failed to change the binding of ³H-GABA to an inhibitor-free crude synaptic membrane preparation, but increased the affinity binding of ³H-GABA to a fresh crude synaptic membrane preparation (see also ref. 14). Freshly prepared membranes revealed Na⁺-independent binding for ³H-GABA with an apparent K_D of ~200 nM (low affinity component)¹⁵. As we have previously reported¹³, the appearance of a high affinity component for ³H-GABA binding in the fresh membrane preparation is prevented by the presence of the endogenous inhibitor. When fresh membranes were preincubated with diazepam (5 × 10⁻⁷ M) there was a change in the kinetic characteristics of the ³H-GABA binding due to the appearance of a high affinity site with an apparent K_D of 36 nM in addition to the low affinity binding component (K_D = 200 nM). Hence, the addition of diazepam to a fresh membrane preparation changes the kinetics of GABA binding in a way similar to that elicited by the removal of the endogenous inhibitor by freezing, thawing washing with Tris buffer and treatment with Triton X-100 as described previously¹³.

The nature of the interaction between the various benzodiazepines and the endogenous inhibitor was then studied by challenging different amounts of the endogenous inhibitor with a constant concentration of benzodiazepines. Figure 2a and b show the effects of 10⁻⁶, 10⁻⁷ and 10⁻⁸ M diazepam and of 10⁻⁷ M Ro-11(+) (a pharmacologically active benzodiazepine, see Fig. 2 legend) and Ro-11(-) (the inactive isomer) on the inhibition of the high affinity ³H-GABA binding induced by the addition of increasing amounts of the endogenous inhibitor to the inhibitor-free membranes. In the presence of benzodiazepines, the amount of inhibitor required to produce an inhibition of the high affinity GABA binding was increased. The difference was particularly significant when small doses of the inhibitor were used. For example, 2.5 µg of the endogenous inhibitor produced ~50% inhibition of the GABA binding in controls or in Ro-11(-)-treated membranes. The same amount of the endogenous inhibitor produced 10, 18 and 32% inhibition in the presence of 10⁻⁶, 10⁻⁷, 10⁻⁸ of diazepam, respectively and 12.5% in the presence of 10⁻⁷ M Ro-11(+). The double reciprocal plot of the data indicates an apparent competitive interaction between the purified endogenous inhibitor and diazepam or Ro-11(+) (see inserts of Fig. 2a and b). The Hill plot analysis of the data gives a Hill coefficient for diazepam- and

Ro-11(+)-treated membranes higher than the Hill coefficient for membranes treated with solvent or Ro-11(-). This confirms that the negative cooperative action of the endogenous inhibitor on ³H-GABA binding is reverted by pretreating the membranes with pharmacologically active benzodiazepines.

We have also tested the potency of a few benzodiazepines to compete with the endogenous inhibitor of GABA binding. Flunitrazepam and clonazepam were four to five times more potent than diazepam but diazepam was at least 10 times more potent than chlordiazepoxide and medazepam. These results suggest that the potency of different benzodiazepines in preventing the inhibition of the high affinity Na⁺-independent GABA binding by the endogenous inhibitor correlates with their binding affinity to saturable sites in rat brain membrane preparations^{8,9} and with the known pharmacological *in vivo* potency as antianxiety agents¹⁶.

The interaction between benzodiazepines and the endogenous inhibitor of Na⁺-independent GABA binding raises the question of whether the endogenous inhibitor interacts with benzodiazepines because it functions as a soluble benzo-

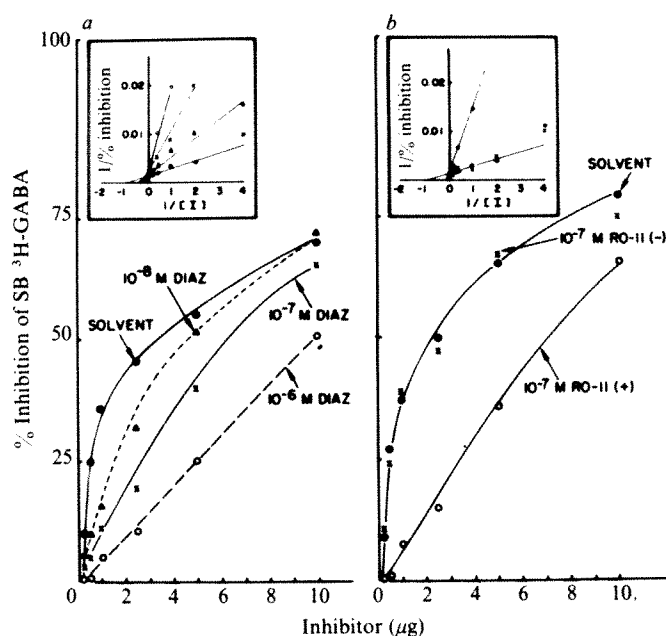


Fig. 2 Interaction of diazepam (a) or Ro-11 (b) with various amounts of endogenous inhibitor of Na⁺-independent ³H-GABA binding. Crude synaptic membranes from rat cerebral cortex were prepared by successive freezing, thawing and washing with Tris buffer plus treatment with Triton X-100 as for Fig. 1. Membranes were preincubated at 0°C for 15 min with diazepam (a) or Ro-11(+) or Ro-11(-) (b) before addition of inhibitor. In the insert double reciprocal plot of the data is shown, revealing an apparent competitive interaction between diazepam, Ro-11(+) and the endogenous inhibitor (I). This gives K values of 0.91 µg of inhibitor for controls, 3.3, 5.2 and 10.2 µg for 10⁻⁸ M, 10⁻⁷ M and 10⁻⁶ M diazepam, respectively, and 9.2 µg of inhibitor protein for 10⁻⁷ M Ro-11(+). Ro-11(+) = Ro-113128; [(+)-5-(*O*-chlorophenyl)-1,3-dihydro-3-methyl-7-nitro-2 H -1,4-benzodiazepine-2-one] is pharmacologically active and Ro-11(-) = Ro-113624 is inactive. Results similar to those reported here were obtained in five experiments with diazepam and in five with Ro-11. The average dose of the inhibitor which produced a 25% inhibition of the GABA binding is 0.5 ± 0.018 µg in 10 preparations of the control membranes, 25% inhibition was achieved with $1.5 \mu\text{g} (\pm 0.082)$, $2.5 \mu\text{g} (\pm 0.10)$ and $4 \mu\text{g} (\pm 0.28)$ of endogenous inhibitor in the presence of 10⁻⁸, 10⁻⁷ and 10⁻⁶ M of diazepam, respectively. A 25% inhibition was achieved with $0.45 \mu\text{g} (\pm 0.020)$ of endogenous inhibitor in the presence of Ro-11(-) and with $3.8 \mu\text{g} (\pm 0.30)$ in the presence of Ro-11(+). The difference between the controls and the Ro-11(-) is not significant. The difference between the controls and different diazepam doses among the different benzodiazepines doses and between Ro-11(-) and Ro-11(+) is statistically significant ($P < 0.05$).

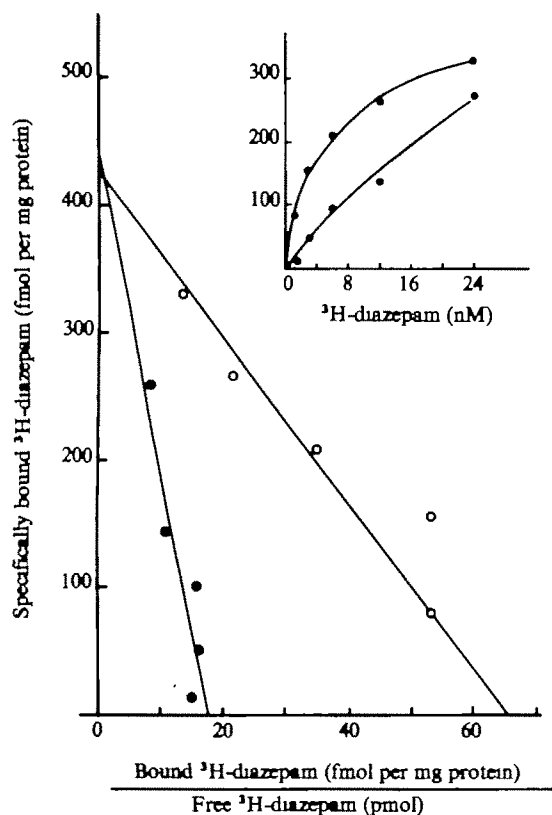


Fig. 3 Scatchard plot of the ^3H -diazepam binding to control or endogenous inhibitor-treated crude synaptic membranes. Frozen, thawed and Triton X-100 treated crude synaptic membranes were prepared as described in Fig. 1. Specific ^3H -diazepam binding was calculated by subtracting the binding which occurred by incubating the membranes at 4°C for 15 min with ^3H -diazepam ($0.75\text{--}24\text{ nM}$; 40 Ci mmol^{-1}), the binding that occurred by incubating with ^3H -diazepam and 10^{-6} M unlabelled diazepam⁹. Concentrations ($0.75\text{--}24\text{ nM}$) of ^3H -diazepam ($100\text{ }\mu\text{l}$) were incubated with a standard aliquot ($200\text{ }\mu\text{g}$ protein) of membranes dispersed in $900\text{ }\mu\text{l}$ of 50 mM Tris-citrate ($\text{pH } 7.1$). The equilibrium binding was terminated by filtration under vacuum through Whatman CF/B glass-fibre filters which were washed twice with 5 ml ice-cold Tris-citrate buffer $\text{pH } 7.1$. When the action of the endogenous inhibitor was studied, the membranes were incubated at 0°C for 60 min in presence of $30\text{ }\mu\text{g}$ of 500-fold purified endogenous inhibitor before addition of ^3H -diazepam. Membranes incubated at 0°C for 60 min without diazepam served as controls. Scatchard analysis gave a K_D of $\sim 6\text{ nM}$ for controls and 24 nM for inhibitor-treated membranes. In the insert, the values of specifically bound (SB) ^3H -diazepam are plotted against the concentrations of ^3H -diazepam in the incubation medium. ○, Controls; ●, inhibitor present

diazepine binding site or as an endogenous modulator of the benzodiazepines for their recognition site in crude synaptic membranes. In fresh membranes (rich in endogenous inhibitor), the extent of diazepam binding is less than in frozen and Triton X-100 treated membranes (free of endogenous inhibitor). Thus, these results exclude the possibility that the endogenous inhibitor acts as a soluble benzodiazepine recognition site.

The data reported in Fig. 3 indicate that the endogenous inhibitor has some of the characteristics expected of a molecule that modulates the affinity of benzodiazepine receptors for this drug. The affinity of ^3H -diazepam for frozen Triton X-100-treated membrane preparations preincubated at 0°C for 60 min with the endogenous inhibitor was reduced when compared to that of similar membranes preincubated without endogenous inhibitor (Fig. 3). The action of the endogenous inhibitor on ^3H -diazepam binding (see Fig. 3) at 0°C was evident only when the endogenous inhibitor was preincubated with the membranes for 15 min or more. Preincubation of crude synaptic membranes with the endogenous inhibitor failed to change the kinetic profile of the recognition site of muscarinic receptors as studied by QNB binding¹⁷.

In summary, the present experiments (compare Figs 1 and 3) indicate that an endogenous inhibitor protein isolated and purified from fresh membrane preparations acts noncompetitively with GABA for its Na^+ -independent recognition site and interacts with diazepam binding. The function of diazepam binding is expressed as a modulation of the affinity of GABA recognition sites. Although binding of diazepam or of endogenous inhibitor modifies GABA binding, they do not exert this effect by binding to GABA binding sites. This regulatory site may be linked to the high affinity site for benzodiazepine binding for which this endogenous inhibitor may act as a modulator or a natural agonist.

This inference agrees with the lack of competition between GABA and ^3H -diazepam for specific binding to brain membranes: moreover, it is in keeping with the relationship between the brain location of ^3H -diazepam and ^3H -GABA binding sites⁹ and is supported by the pharmacological interactions between diazepam and GABA receptor blockers^{1,3}. The apparent decrease in the affinity for ^3H -diazepam binding in brain synaptic membranes pretreated with the endogenous inhibitor could suggest that the endogenous inhibitor is functioning as a natural ligand for the benzodiazepine receptors. However, interpretation of these data requires caution because the time constants of our measures are not sufficiently short. In fact, we are dealing with a very complex system in which the theoretical assumption of a rapid reversible equilibrium implied by any inference derived from the Scatchard analysis may not be satisfied. Although our data clearly indicate that the 'endogenous inhibitor' may act as an 'endogenous modulator' of the benzodiazepine binding, it is at present impossible to establish whether this occurs as a result of a direct action at the high affinity receptor sites for the benzodiazepines or indirectly through an allosteric effect which involves the GABA/benzodiazepine/ Cl^- ionophore supramolecular complex. Studies are now in progress to elucidate this problem. These studies may also help to clarify the supramolecular nature of the postsynaptic GABA receptors and the role of the endogenous inhibitor protein in the regulation of the affinity of ^3H -GABA for the Na^+ -independent GABA recognition site. Preliminary work in our laboratory indicates that diazepam displaces the endogenous protein inhibitor from its physiological binding sites also *in vivo*. Whether a change in the amount of this endogenous inhibitor bound to synaptic membranes plays a part in determining the level of anxiety is not yet known but this can now be investigated.

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Is there a correction mechanism in the 5S multigene system?

RIBOSOMAL GENES are present in many copies per cell in all living species. They are the classic example of the tandemly repeated multigene system, and have been studied in a wide variety of organisms, including man^{1,2}, *Drosophila*^{3,4}, *Xenopus*^{5,6} and *Escherichia coli*⁷. Reiteration of these genes is presumably necessary to ensure the production of the large amounts of ribosomal and 5S RNA required for use in the ribosomes. However, the possession of multiple copies of a gene poses a special problem for evolution. If a lethal or deleterious mutation occurred in one gene of, for example, the 24,000 present for the 5S DNA in *Xenopus*⁵, this would have no selective disadvantage for the organism. What then prevents the accumulation of lethal mutations in such a system? Presumably if the number of effective genes were reduced significantly natural selection would operate. But it has been suggested that a 'correction mechanism'⁸ exists to maintain the homogeneity of the multigene system without a requirement for natural selection. We show here that two cloned repeats of *Xenopus* oocyte 5S DNA are very similar but not identical in sequence. The similarity strongly suggests that a correction mechanism is operating, but it is not so precise as to cause the different repeats to be identical. A small but distinct degree of heterogeneity is tolerated.

Two main kinds of correction mechanism have been proposed—'sudden' and 'gradual'. The master-slave hypothesis⁹ (a sudden mechanism) envisages the matching of a number of 'slave' sequences to a single 'master' once per life cycle. Thus neighbouring repeats or slaves should be identical. In the oocyte 5S DNA of *Xenopus laevis* adjacent repeating units are clearly heterogeneous in length¹⁰, ruling out this mechanism. A gradual mechanism called 'crossover fixation' has been proposed by Smith¹¹ and envisages a high rate of unequal crossing over, causing the fixation of multiple copies of a single ancestral repeat. This seems a more plausible theory, but the extent to which it operates is uncertain because the real extent of correction or homogeneity between the members of a multigene system has never been absolutely clear.

We have examined the extent of correction in detail for the oocyte 5S DNA gene system in *Xenopus laevis*. This DNA consists of a high A+T rich spacer made up of many short subrepeats followed by a G+C rich region which is a duplication of 174 nucleotides, containing the 5S gene and also a 'pseudogene' structure. The overall organisation of a repeat unit is shown in Fig. 1. Previous work on the oocyte 5S RNA¹²⁻¹⁴ has shown that it is a mixture of molecules, but the real extent of heterogeneity could not be assessed accurately¹³. Recently the entire sequence of total frog 5S DNA was determined^{15,16} but, again, microheterogeneity remained undetected, because a mixture of sequences was studied. We have compared the entire sequence of two cloned repeat units of oocyte 5S DNA, Xlo 5

and Xlo 31. These are by definition unique species, so that the sequences obtained must actually exist and are not averaged¹⁷.

The sequence of these repeat units was determined by the Maxam and Gilbert technique¹⁸. Typical sequencing gels are shown in Fig. 2. The complete sequence of clone Xlo 5 is shown in Fig. 3 with the differences present in clone Xlo 31 indicated. Two types of microheterogeneity were found. The first involved the addition or deletion of oligonucleotide blocks in the A+T rich spacer region. This phenomenon has been previously noted

Fig. 2 Two thin gels¹⁹ showing different parts of the sequence of the 193-long *Hae*III fragment of Xlo 5. Missing residues denoted by X are methylated Cs situated in *Eco* RII restriction methylation sites¹⁶.

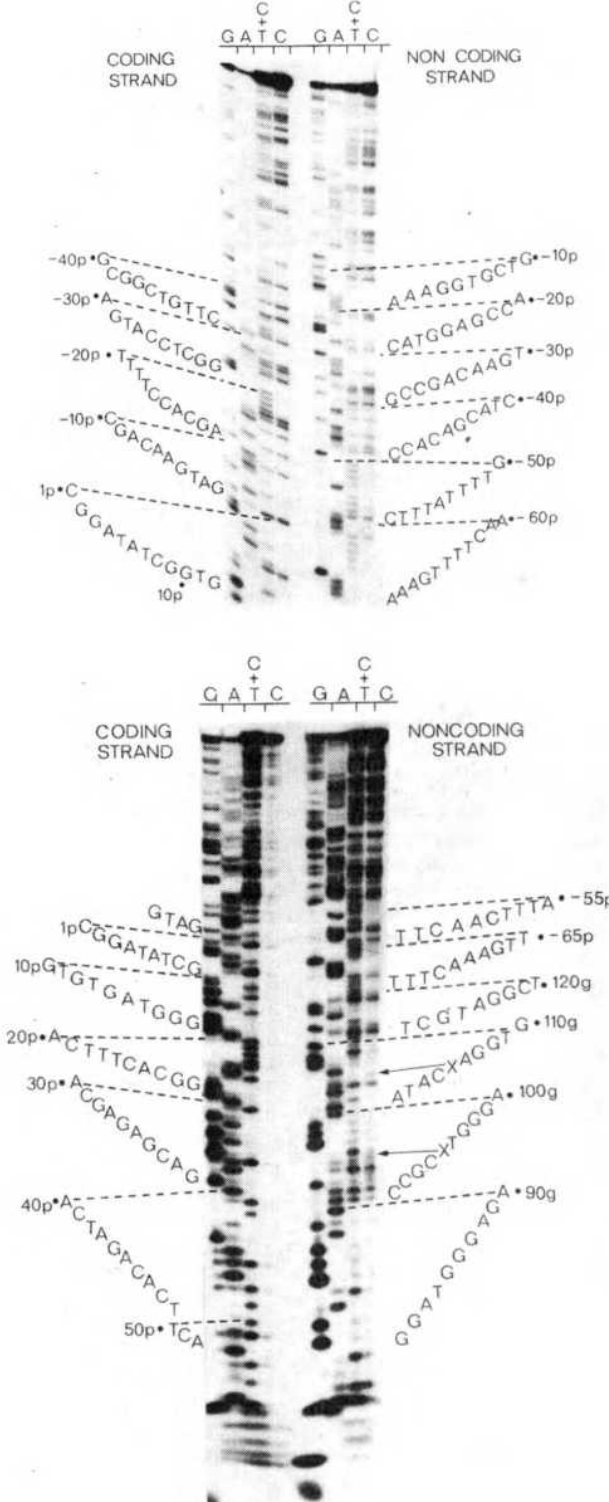
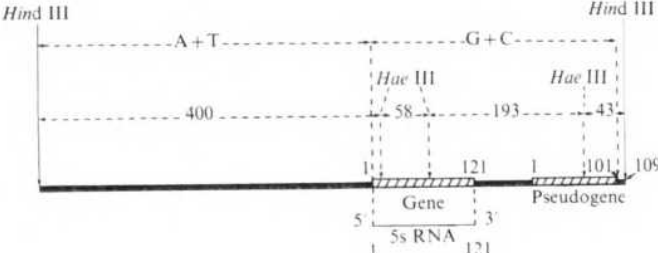


Fig. 1 Location of gene and pseudogene and the *Hae*III and *Hind*III sites in the 5S DNA monomer.





The concept of unequal crossing over is compatible with the observed sequence microheterogeneity. As noted previously¹⁵ the presence of the 15-long oligonucleotide within a larger repeat structure correlates well with the prediction of the development of short and long range periodicities within the A+T spacer region¹¹. We must now assume that natural

selection will eliminate mutations in the promoter regions, whereas a level of heterogeneity in the spacer and gene regions can be tolerated. The extent to which the correction mechanism works on the gene sequences need only be enough to keep the heterogeneity of the 5S RNA in ribosomes within a limit which the animal can tolerate. As the precise function of 5S RNA is unknown, this limit is unclear, but is perhaps not more than a few mutations per the 'averaged' 5S RNA sequence'.

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Splicing of SV40 late mRNA is a post-transcriptional process

THE synthesis and metabolism of SV40 mRNA is being used as a model system to study RNA transcription and processing in animal cells. We and others have recently reported the finding of a novel mechanism of viral RNA maturation in SV40-infected cells¹⁻⁷ in which the leader sequences (those found at the 5' ends of the main bodies of SV40 late mRNAs) and the adjacent coding sequences are transcribed from non-contiguous segments of the viral DNA. Similar observations have been reported for adenovirus mRNAs^{8,9}. This process is known as splicing and may proceed by several possible routes. These include: intermolecular ligation of RNA; deletion in the DNA template of the intervening sequences; looping out of intervening DNA sequences so that the RNA polymerase could skip over short distances; and deletion of the appropriate intervening RNA sequences at the post-transcriptional level. We present here an analysis of R-loop structures¹⁰ formed between nuclear and cytoplasmic poly(A)⁺ RNAs and linear SV40 DNA, which provides support for the last of these possibilities⁴⁰. A similar analysis has been carried out for β -globin mRNA¹⁷.

It has been shown that nuclear poly(A)⁺ viral RNA accumulates in the nucleus of the infected cell (refs 9, 10 and Y.A., M.H., O.L. and Y. Reuveni, submitted for publication). Figure 1 shows that the majority of the poly(A)⁺ viral RNAs present in the nuclei of infected cells sediment in sucrose gradient as 19S components: only a minor fraction sediments faster than 19S and no 16S poly(A)⁺ viral RNA is observed.

R-loops were obtained by annealing nuclear or cytoplasmic 19S poly(A)⁺ RNA with double-stranded linear SV40 DNA obtained by cleavage of form I DNA with *Bgl*I or *Taq*I restriction endonucleases. *Bgl*I and *Taq*I cleave form I DNA at 0.67 and 0.58 map units respectively¹. Figures 2 and 3 show the appearance of representative R-loop structures with their schematic tracings. There are two striking differences in the appearance of the R-loops: (1) the 5' ends of the R-loops pertaining to the nuclear RNAs are closer to the restriction

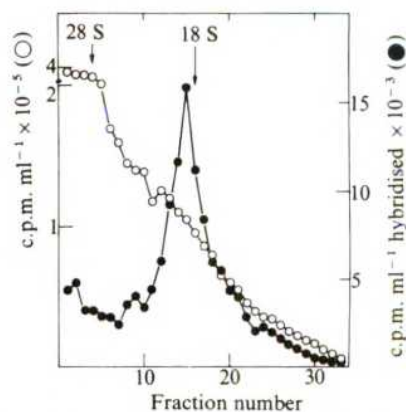
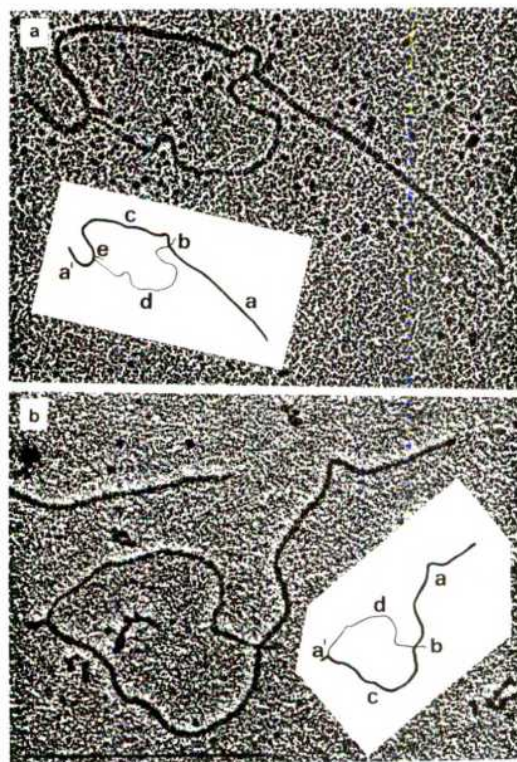


Fig. 1 Sedimentation analysis of nuclear poly(A)⁺ viral RNA. Forty-eight hours after infection of BSC-1 cells with plaque-purified SV40 stock (strain 777), cells were labelled with ³H-uridine for 5 h and labelled nuclear RNA extracted¹³. The ³H-RNA was dissolved in 1 ml of 0.5 M NaCl, 0.01 M Tris-HCl pH 7.4 and fractionated on an oligo-dT-cellulose column (0.5 × 3 cm) as described before¹⁴. Poly(A)⁺ RNA was eluted from the column with 0.01 M Tris-HCl pH 7.4 and collected by ethanol precipitation and centrifugation. Sedimentation analysis of the labelled RNA was performed in 15-30% (w/w) sucrose gradient in SDS buffer¹⁵, centrifuged for 16 h at 25,000 r.p.m. at 20°C in a Spinco SW27 rotor. At the end of the run, fractions were collected and the radioactivity counted in Triton-based scintillation fluid. Twenty-five-microlitre aliquots from each fraction were hybridised to SV40 DNA immobilised on nitrocellulose filters in 4 × SSC at 68°C for 20 h, as previously described¹⁰. Fractions 10-20 were collected and used for the R-loop analysis.

Fig. 2 Electron micrographs and their tracings of R-loops composed of linear SV40 DNA (form I cleaved at position 0.67 map units with *Bgl*I restriction endonuclease)¹ and cytoplasmic 19S poly(A)⁺ RNA in *a* or nuclear poly(A)⁺ RNA in *b*. Preparation of the R-loops for electron microscopy was as detailed in ref. 3. In the tracing the letters represent: *a*, double-stranded DNA; *b*, free tail of poly(A); *c*, a heteroduplex DNA-RNA which includes the body of the RNAs; *d*, displaced single-stranded DNA; *e*, free tail of a 'leader'³; *a'*, double-stranded DNA. See text for differences between R-loops pertaining to nuclear and cytoplasmic 19S poly(A)⁺ RNAs. Scale bars = 0.5 μ m.



enzyme cleavage sites than are the 5' ends of those pertaining to the cytoplasmic RNAs; (2) free leaders are often seen at the 5' ends of the R-loops pertaining to the cytoplasmic 19S RNA (in about 70% of the molecules), whereas they are observed much less frequently in the R-loops pertaining to the nuclear 19S viral RNA (in about 10% of the molecules).

Figure 4 is a histogram summarising the map positions of the 5' ends of nuclear and cytoplasmic 19S poly(A)⁺ RNAs. The position of the 5' termini of the cytoplasmic RNA species (dark histogram) is represented by a reasonably sharp histogram with a mean at coordinate 0.77 ± 0.01 . The histogram for the location of the 5' termini of the nuclear viral RNAs (striped histogram) is skewed towards an upstream position and is more heterogeneous. The apparent sharpness of the histogram in nuclear RNA/SV40 DNA (*Bgl*I) (Fig. 4a) results presumably from the fact that the 5' ends of the R-loops are close to the *Bgl*I cleavage site and are therefore less stable. Indeed, 'opened' forked R-loops were observed in this preparation. The small peak at coordinate 0.95 represents a minor contamination with cytoplasmic 16S RNA which is the predominant viral RNA accumulating in the infected cells. The heterogeneity of the location of the 5' ends of nuclear viral RNAs is consistent with a model previously suggested for transcription of prokaryotic DNA¹⁶. Accordingly, several RNA polymerases bind to a short stretch of DNA from which they are released in succession to begin transcription.

The ability of the precursor nuclear poly(A)⁺ viral RNA to anneal to the entire length of the late portion of SV40 DNA (~0.67–0.17 map units) including the corresponding interruptions between the leaders and the bodies of the cytoplasmic mRNAs indicates that splicing is a post-transcriptional process. Splicing of the precursor poly(A)⁺ viral RNA may occur in the nucleus before the RNA molecule is transported to the cytoplasm. It is possible, however, that splicing occurs during the transport from the nucleus to the cytoplasm or immediately after the molecule has reached the cytoplasm. The excision–ligation

Fig. 3 Electron micrographs and their tracings of R-loops composed of linear SV40 DNA (form I cleaved at position 0.58 map units with *Taq*I restriction endonuclease)¹ and cytoplasmic 19S poly(A)⁺ RNA in *a* or nuclear poly(A)⁺ RNA in *b*. See Fig. 2 for explanations.

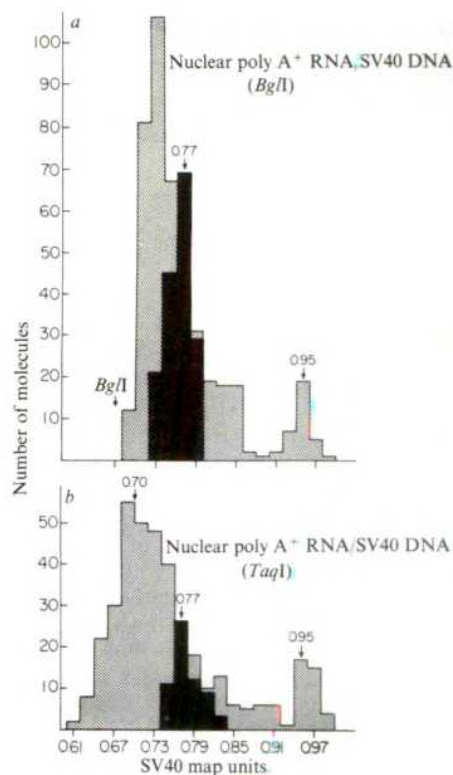
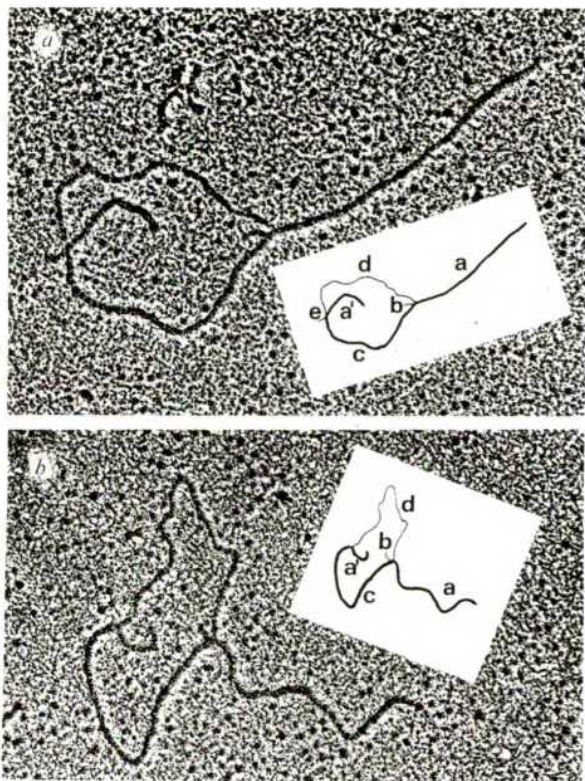


Fig. 4 Histogram of the locations, on the physical map of SV40, of the 5' ends of the bodies of the 19S poly(A)⁺ nuclear and cytoplasmic viral RNAs. The locations of the 5' ends of the bodies of the viral RNAs were determined from measurements of molecules as in Figs 2 and 3. The distances between the 5' end of the R-loop and the restriction enzyme cleavage site (*a*' in the tracing) were measured and expressed for each molecule as the fractional length of the viral DNA. The locations on the physical map of SV40 DNA were determined by adding the value obtained to 0.67 in *a* and to 0.58 in *b*. The dark histogram is cytoplasmic RNA and the striped histogram is nuclear RNA.

reaction in the precursor RNA molecule is carried out, presumably, by an enzyme(s) which recognises short base-paired sequences in the precursor RNA molecule. This reaction would represent a novel mechanism in the regulation of gene expression in animal cells.

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3'-Terminal labelling of RNA with T4 RNA ligase

T4 RNA LIGASE catalyses the formation of an internucleotide phosphodiester bond between an oligonucleotide donor molecule with a 5'-terminal phosphate and an oligonucleotide acceptor molecule with a 3'-terminal hydroxyl¹⁻³. Although the minimal acceptor must be a trinucleoside diphosphate, dinucleoside pyrophosphates and mononucleoside 3',5'-bisphosphates (pNps) are effective donors in the intermolecular reaction⁴⁻⁶. We demonstrate here that various high molecular weight RNA molecules are acceptors in the RNA ligase reaction even when present in very low concentrations in the reaction mixture. One immediate consequence of this observation is that a convenient method for labelling the 3' end of RNA molecules *in vitro* becomes available. By using a [5'-³²P]pNp as a donor and RNA as an acceptor, the product of the reaction is an RNA molecule one nucleotide longer, with a 3'-terminal phosphate and a ³²P-phosphate in the last internucleotide linkage. This reaction is therefore analogous to the *in vitro* labelling of the 5' termini of RNA chains with polynucleotide kinase and [γ -³²P]ATP and can be used in situations where 5' labelling is not possible. In addition, the ability to add various donors to an RNA molecule should allow the function of the 3' terminus of the molecule to be investigated.

Twelve different purified preparations of one or more RNA species were tested for their ability to act as acceptors in the RNA ligase reaction (Table 1). These RNAs varied in chain length from ~870 nucleotides (smallest of the 10 segments of the reovirus genome⁷) to ~15,000 nucleotides (Sendai virus RNA⁸). [5'-³²P]pAp or pCp, the donors in these reactions, were prepared from [γ -³²P]ATP and the corresponding 3' monophosphate using T4 polynucleotide kinase. In extensive studies with oligomers⁵ and tRNA⁹, pCp is found to be the more reactive donor and is, therefore, preferable for 3'-end labelling.

Table 1 3' End labelling of RNA

RNA (source)	Concentration of acceptor in reaction (nM)	Yield (%)
<i>E. coli</i> 16S rRNA (C. Cantor)	120	42
<i>E. coli</i> 23S rRNA (D. Shapiro)	40	18
<i>E. coli</i> 23S + 16S rRNA (S. Rose)	200	44
Rabbit reticulocyte 18S rRNA (D. Shapiro)	110	24
Human 28S rRNA (B. Coté)	40	39
Satellite tobacco necrosis virus (STNV) RNA (J. Clark, Jr)	180	93
Brome mosaic virus RNA 1-4 (P. Kaesberg)	85	77
Brome mosaic virus RNA 4 (P. Kaesberg)	220	54
Q β virus RNA (P. Cole)	60	25
Sendai virus RNA (D. Kolakofsky)	12	107
Reovirus RNA (A. Shatkin)	130	8
Ovalbumin mRNA (D. Shapiro)	100	114

Reactions were carried out in a total volume of 30 μ l in the following conditions: 1 μ g RNA (12–220 nM), [5'-³²P]pAp (1 μ M, 850–1,520 Ci mmol⁻¹), ATP (5 μ M), HEPES (50 mM, pH 8.3), MgCl₂ (10 mM), dithiothreitol (3.3 mM), dimethyl sulphoxide (10%, v/v), glycerol (15%, v/v) and RNA ligase (400 units ml⁻¹, 200 μ g ml⁻¹), and incubated at 4 °C for 24 h. (The RNAs were provided by those named in parentheses.) Yields were determined from the amount of radioactivity precipitable on a nitrocellulose filter in 5% trichloroacetic acid (TCA), 100% reaction equalling the number of mols of RNA added. However, as several samples were contaminated with smaller RNAs their values generally represent an overestimate of the yield. For example, high levels of incorporation were not expected for STNV RNA, as about 50% of the 3' ends are blocked with a phosphate¹⁸. Analysis on a 20% acrylamide gel indicated that the majority of TCA-precipitable label was incorporated into oligomers less than 80 nucleotides in length.

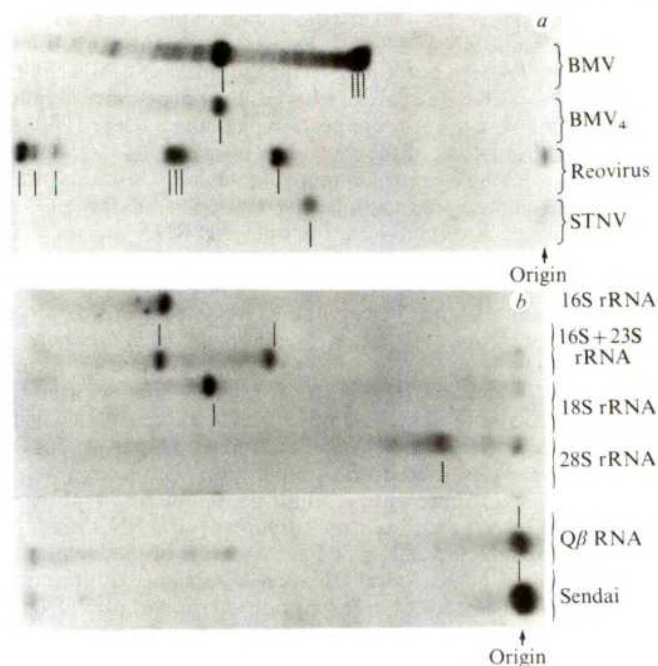


Fig. 1 Analysis of RNA ligase reactions by vertical, denaturing polyacrylamide gel electrophoresis. Aliquots from reactions in 9 M urea-glycerol (1:1, v:v) were loaded on to acrylamide slab gels (19:1, acrylamide:bisacrylamide; 3 mm $\times$ 22 cm) containing 7 M urea, 90 mM Tris-borate (pH 8.3) and 10 mM EDTA¹⁹ and electrophoresed at 250 V at 25 °C with the borate-EDTA as running buffer. In adjacent wells, 1–3 μ g of the unreacted RNAs were run. Radioactive bands were visualised by autoradiography. The lines indicate the position of the unreacted RNAs visualised with 'Stains-All'. a, BMV₍₁₋₄₎, BMV₄, reovirus and STNV were electrophoresed for 16 h on a 4% acrylamide gel. b, 16S rRNA, 23S plus 16S RNA, 18S rRNA, 28S rRNA, Q β and Sendai were electrophoresed for 20 h on a 2.5% acrylamide gel.

Each reaction contained 1 μ g of RNA, a substantial excess of donor and ATP, and a high concentration of RNA ligase (5 μ M). A low temperature (4 °C) and long incubation time (> 16 h) were chosen to minimise the rapid loss of RNA ligase activity which occurs at higher temperatures^{5,10}. Finally, we have found that the extent of reaction is increased two- to threefold⁵ by including 10% dimethyl sulphoxide in the reaction mixture.

Analysis of the yields by acid precipitation of the RNA and Millipore filtration indicated that although all 12 RNA preparations were acceptors, the efficiency of the reaction varied considerably. Differences in the concentration of 3' termini cannot be the only cause for this, as no correlation between yield and concentration was observed. The sequence and the secondary structure at the 3' end of the RNA are also thought to affect the reaction yield. Comparison of oligonucleotide acceptors indicates that the composition of the terminal three nucleotides has a strong influence on yield⁵. The known specificity of RNA ligase for single-stranded RNA¹¹ can account for the low yield encountered for the 'blunt', double-stranded end of reovirus RNA and suggests that a 3'-terminal secondary structure could explain the comparatively low yields with other RNAs.

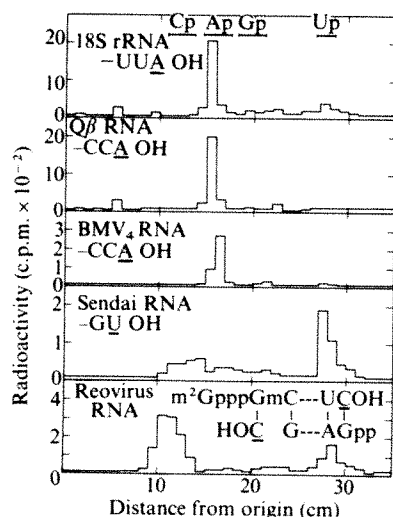
In Fig. 1, the products of the reactions in Table 1 are analysed by acrylamide gel electrophoresis in 7 M urea, where the secondary structure of RNA is minimised. Autoradiography of the gels reveals that the major ³²P-labelled RNA bands migrate closely with the major staining bands. This observation supports the conclusion that the [5'-³²P]-labelled nucleotide bisphosphate has been added on to the 3' end of the RNA. When a mixture of RNA molecules was used in a reaction (*Escherichia coli* rRNA, brome mosaic virus (BMV) and reovirus) all species were labelled to a similar extent. In addition, contaminating RNAs present in the reaction mixture are also labelled. In several of these cases, the smaller contaminants have run off the end of the gel and can only be detected with higher percentage

gels. However, we can exclude fragmentation of the product during the ligation reaction, as smaller RNAs are not observed in all cases.

In several of the reactions in Table 1, the ^{32}P -labelled RNA products have been purified from the unreacted bisphosphate and any low molecular weight impurities by Sephadex G-100 column chromatography. Complete digestion of these RNAs with a mixture of ribonucleases A, T_1 and T_2 and analysis of the products by high-voltage paper electrophoresis give a single ^{32}P -labelled nucleoside monophosphate corresponding to the known 3'-terminal nucleotide of the RNA molecule (Fig. 2). In the case of double-stranded reovirus RNA, analysis of a T_1 digest of the labelled RNA indicates that the 3' ends of both the plus and the minus strands were being labelled to a similar extent (A. Shatkin, personal communication). Finally, it is important to note that no reaction occurs at the 3' hydroxyl of m⁷G of the 'cap' structures on reovirus RNA, BMV RNA or ovalbumin mRNA. It is unlikely that cap structures will be substrates for RNA ligase, as a trinucleoside diphosphate is required as a minimal acceptor^{2,5} and isolated Ado5'ppp5'Guo was found to be inactive as a donor⁴.

The use of T4 RNA ligase to label the 3' end of RNA *in vitro* offers several advantages over other methods of *in vitro* labelling of RNA. First, the 3' ends of RNA molecules are usually free 3' hydroxyl groups and therefore can be used directly as acceptors for RNA ligase. In contrast, labelling the 5' ends of RNA with T4 polynucleotide kinase is often complicated by the presence of one or more 5'-terminal phosphates or the 'cap' structure, which must be removed before the labelling reaction can be carried out. Second, as endonucleolytic breakdown products of RNAs will often have a 3'-terminal phosphate and a 5'-terminal hydroxyl, they will be labelled by polynucleotide kinase but not by RNA ligase. Thus, RNA ligase reactions will have fewer radiolabelled contaminants. Third, in contrast to other *in vitro* procedures that are limited to a single isotope^{12,13}, nucleoside 3',5'-bisphosphates may be prepared as 5'- ^{32}P or with ^3H or ^{14}C in the nucleoside. (^{125}I)5-iodocytidine 3',5'-bisphosphate is also a donor in the RNA ligase reaction⁹. Finally, as preparations of RNA ligase can be made free of nucleases and the reaction conditions are extremely mild, the labelled RNA can be recovered intact in high yields.

Fig. 2 Nearest-neighbour analysis of 3'-end labelled RNA. The RNAs from ligation reactions were purified by gel filtration on Sephadex G-100, ethanol-precipitated and degraded completely with a mixture of RNases A, T_1 and T_2 according to Barrel²⁰. The 3' mononucleotides were resolved by paper electrophoresis on Whatman no. 1 in 0.025 M Na citrate, pH 4.0, at 4,500 V for 2 h (ref. 21). The sequences of the 3' termini of 18S rRNA, Q β , BMV₄, Sendai and reovirus have been reported elsewhere (refs 22, 23, 17, 24, 25, respectively).



RNA with a radiolabel at the 3' end may be used to determine the sequence of nucleotides near the label by one of several recently developed methods¹⁴⁻¹⁶. For example, 15 nucleotides of the 3'-terminal sequence of BMV-4 RNA were confirmed¹⁷ with material prepared in this work by using the two-dimensional 'wandering-spot' sequencing procedure (R. Dasgupta and P. Kaesberg, personal communication). Thus, 3'-terminal labelling with RNA ligase complements 5'-terminal labelling with polynucleotide kinase, and the two methods may be used to study the sequence at both ends of a molecule and, if the molecule is short enough, confirm the results of the other method.

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Cloning of replication origins from the *E. coli* K12 chromosome

MINIPLASMIDS, containing only an origin of replication and plasmid-coded functions necessary for plasmid replication and maintenance have been isolated from larger plasmids¹. The technique used was to cleave the parent plasmid with the restriction endonuclease *EcoRI* and reanneal and ligate the fragments so generated in the presence of another *EcoRI*-generated fragment of DNA coding for a β -lactamase. This second fragment was not capable of self-replication but hybrids between it and a plasmid fragment which was able to replicate could be detected because calls of *Escherichia coli* transformed with such hybrids would be resistant to ampicillin. We have used the same technique to isolate minichromosomes from the total chromosomal DNA of *E. coli* K12 and have found that two different chromosomal *EcoRI* fragments can function as autonomously replicating plasmids. One type of plasmid is indistinguishable from that isolated independently by Yasuda and Hirota² and contains the origin of replication normally active in chromosome replication termed *oriC* (refs 3-6). The other type, which we call *oriJ* plasmids to distinguish them from

oriC plasmids, are described below. Both types can be isolated from a single strain of *E. coli* and they contain chromosomal DNA which is not homologous in DNA-DNA hybridisation studies. Consequently we conclude that the *E. coli* K12 chromosome contains at least two segments of DNA capable of self-replication.

The fragment of DNA coding for β -lactamase (the Ap fragment) used in our study was obtained from an amplifiable ColE1-Ap plasmid (pLG1) which was constructed by *in vivo* ligation of the Ap fragment (4.5 megadaltons) of the plasmid pSC122 (ref. 1) with ColE1 DNA which had been cleaved at its single *EcoRI* site. ColE1-Ap amplified by incubation of its host in chloramphenicol⁷ was purified⁸ and cleaved with *EcoRI* and the Ap fragment separated in an equilibrium density gradient of CsCl, making use of a density difference between it ($\rho = 1.693$) and the linear form of ColE1 ($\rho = 1.710$) (ref. 7).

In one experiment, an Ap fragment preparation (1 μ g) was annealed and ligated⁹ with an *EcoRI* digest of *E. coli* K12 DNA (3 μ g) isolated from strain C600 (ref. 10) by Marmur's method¹¹. After dialysis, the ligation mixture was used to transform¹³ the F⁻ *recA* strain AB2463 (ref. 12), and ampicillin resistant transformants were selected on appropriately supplemented M9 synthetic medium¹⁴ containing ampicillin (40 μ g ml⁻¹). Before plating the transformed cells were incubated in LB broth¹⁵ for 90 min at 37 °C to permit expression of the β -lactamase gene. Of 1,600 ampicillin-resistant colonies which emerged, 1,000 were tested and 5 found to be sensitive to colicin E1. Three of these gave ampicillin-sensitive segregants, and were found to contain plasmid DNA when total lysates¹⁶ were analysed in CsCl-ethidium bromide equilibrium density

gradients¹⁷. DNA isolated from C600, AB2463, and a pooled extract from 10 ampicillin-sensitive segregants of one ampicillin resistant clone, contained no detectable plasmid DNA.

Each of the three plasmids from the ampicillin-resistant, colicin-sensitive clones gave two *EcoRI* cleavage fragments, one of 4.5 megadaltons corresponding to the Ap fragment and another of 8.3 megadaltons. They seemed to be identical on the basis of subsequent analysis with *HindIII*. One of them, called *oriJ*-Ap (pLG2), was subjected to further analysis. Interestingly, none of these first three plasmids had an *EcoRI* fragment of 5.9 megadaltons as reported for the *oriC* plasmids. The 8.3 megadaltons fragment is not a derivative of ColE1 (4.2 megadaltons), the only other replicon known to be involved in our protocol, and is not derived from contaminating DNA. Both alternatives were ruled out by DNA-DNA hybridisation using a modification¹⁸ of Southern's¹⁹ method. *OriJ*-Ap DNA was labelled to high specific activity with ³²P by nick-translation¹⁸. This DNA was then used as a probe to test for hybridisation against *EcoRI*-digested ColE1 DNA, Ap DNA, *EcoRI*-digested *oriJ*-Ap DNA, an *EcoRI* digest of *E. coli* DNA from strain C600 and an *EcoRI*-digest of bacteriophage λ C1857 DNA. No hybridisation occurred between ColE1 and *oriJ*-Ap (Fig. 1a, b) indicating that they contain no extensive sequence homology and thus that the latter is not derived from the former. As a control it can be seen that both *EcoRI* fragments of *oriJ*-Ap (Fig. 1e, f) and the Ap fragment (Fig. 1c, d) hybridised with *oriJ*-Ap DNA. Figure 1g, h shows that *E. coli* DNA contains an *EcoRI* fragment 8.3 megadaltons in size which hybridises with *oriJ*-Ap. On the other hand (data not shown) the Ap fragment itself shows no hybridisation with *E. coli* DNA, hence the hybridisation seen in Fig. 1h must be between *E. coli* DNA and the 8.3 megadalton fragment of *oriJ*.

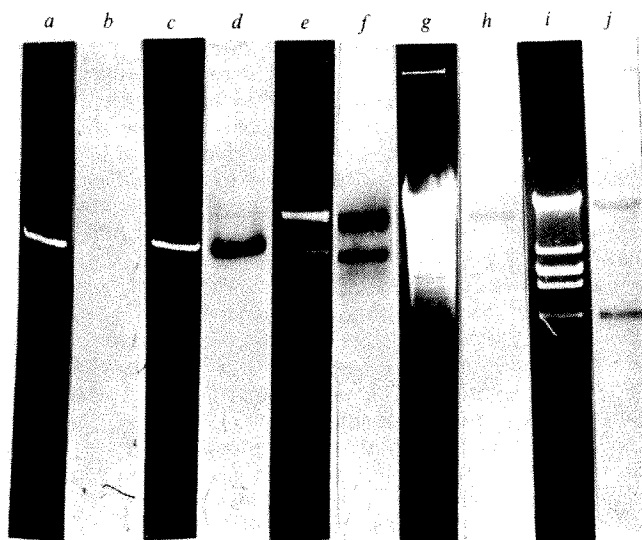
The two *EcoRI* fragments of bacteriophage λ C1857 DNA which contain the cohesive ends²⁰ gave a faint reaction with the *oriJ*-Ap probe but no hybridisation was detected with the 4.7-megadalton fragment which contains the origin of phage replication²⁰ (Fig. 1, track j). Note that in Fig. 1 the exposure time for track j was 8 times longer than for other tracks. The significance of the faint reaction is not clear but the absence of homology with the 4.7-megadalton fragment suggests that *oriJ* is not derived from λ DNA which might be present in the C600 chromosome.

A further study of the structure of *oriJ*-Ap was made by a combination of restriction analysis with *EcoRI* and *HindIII* enzymes and hybridisation with a ³²P-labelled Ap DNA probe. The results obtained are shown in Fig. 2 and confirm that the 4.5-megadalton *EcoRI* fragment of *oriJ*-Ap is the Ap fragment used in the cloning experiment, and are consistent only with the restriction map shown in Fig. 2B or with one in which the order of the 2.4- and 1.4-megadalton segments is reversed. Restriction analysis with *Bam*HI enzyme (data not shown) confirm the order given in Fig. 2B.

To compare the restriction map of *oriJ* with that of the homologous 8.3-megadalton fragment of C600 chromosomal DNA, ³²P-labelled *oriJ*-Ap was hybridised against C600 DNA digested with *EcoRI*, *HindIII* or both endonucleases (Fig. 2C). An analysis of the molecular weights of the major hybridisation bands shows that the 8.3-megadalton fragment present in the C600 chromosome generates a family of *HindIII* fragments indistinguishable from the fragments generated by the 8.3-megadalton component of *oriJ*-Ap. Thus it seems that a single segment of chromosomal DNA has been incorporated into *oriJ*-Ap probably without major structural rearrangements. The data in Fig. 2C also provide information about the location of the two *HindIII* cleavage sites adjacent to the 8.3-megadalton segment and these are indicated in Fig. 2D.

It is conceivable that different K12 strains have different restriction target maps in the neighbourhood of the chromosome origin and that *oriJ* and *oriC* both contain the same origin of replication. Strain KL228 (ref. 21) is an Hfr from which F-prime derivatives have been isolated that carry a 5.9-megadalton *EcoRI* fragment inside a restriction map which is

Fig. 1 Homology studies with *oriJ*-Ap DNA. *OriJ*-Ap (0.5 μ g) was labelled with ³²P (1–5 $\times 10^6$ cpm μ g⁻¹) by nick-translation¹⁸. This probe was hybridised^{18,19} to DNA (0.1–0.15 μ g) that had been previously digested to completion with *EcoRI*, fractionated by electrophoresis (90 min at 150 V) in 0.8% agarose containing Tris-borate-EDTA buffer²⁹, denatured in the gel by treatment with alkali and finally blotted onto nitrocellulose filters. Hybridisation was carried out in 3 $\times$ SSC at 65 °C for 2 d. The filters were given a post-hybridisation wash in 3 $\times$ SSC followed by 0.1 $\times$ SSC at 65 °C. Hybridisation bands were detected by autoradiography using a Kodak X-Omat film that was exposed to the filters at –80 °C for 18 h (tracks b, d, f, h) or 6 d (track j). The ethidium-bromide pattern (left) and the corresponding DNA material that hybridises with the probe (right) are shown in pairs: tracks (a) and (b) ColE1 DNA $\times$ *EcoRI*, (c) and (d) Ap fragment, (e) and (f) *oriJ*-Ap $\times$ *EcoRI* (control), (g) and (h) C600 DNA $\times$ *EcoRI* and (i) and (j) λ C1857 DNA $\times$ *EcoRI*. (Molecular weights of λ fragments: 13.7, 4.7, 3.7, 3.5, 3.0 and 2.0 megadaltons respectively; 3.7 and 3.5 megadalton fragments are not resolved in the gel).



indistinguishable from that of the 5.9-megadalton fragment of *oriC* (ref. 4). When *oriJ*-Ap was used as a probe to test for hybridisation with an *EcoRI* digest of DNA from KL228, no hybridisation was found in the 5.9-megadalton region (Fig. 2C, track d). On the contrary, the hybridisation pattern against the *EcoRI* digest and a *HindIII* digest (Fig. 2A, d and e) of this DNA indicates that it contains the *oriJ* segment. Thus KL228 probably contains both the *oriC* and *oriJ* segments.

If the two plasmids are derived from distinct regions of the K12 chromosome then both should be recoverable in a single DNA preparation. The fact that none of the three ampicillin resistant clones isolated by us contained *oriC* plasmids whereas all three such clones isolated in a similar way by Yasuda and Hirota did so, suggested that the choice of recipient strain might

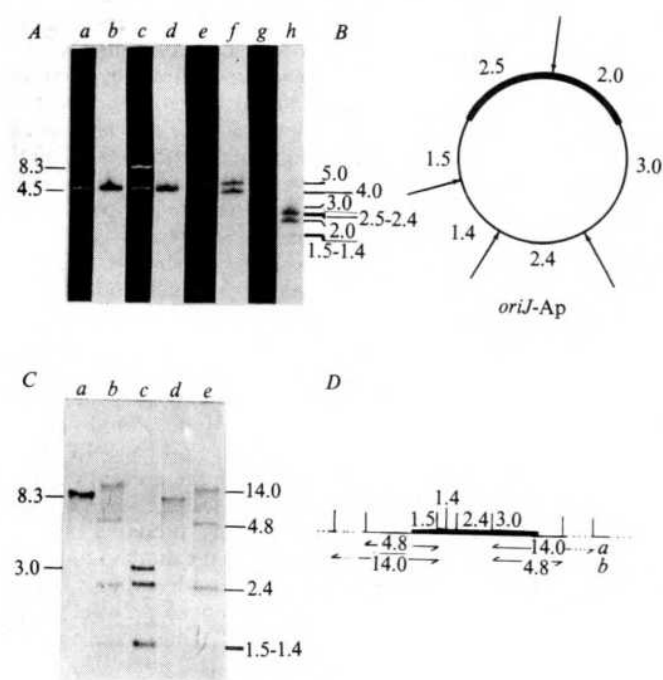
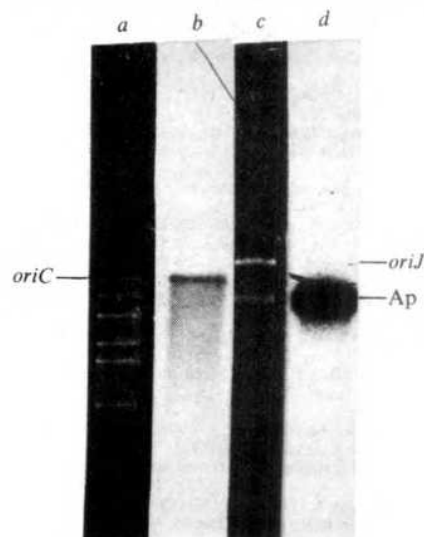


Fig. 2 Physical analysis of *oriJ*-Ap and of the homologous *E. coli* K12 chromosomal region. **A**, Combined restriction and hybridisation analysis of *oriJ*-Ap. *oriJ*-Ap (0.3 μ g) was digested with *EcoRI*, *HindIII* or both enzymes, and the products fractionated by electrophoresis in agarose gel and hybridised with Ap DNA (0.5 μ g) labelled with ^{32} P as indicated in Fig. 1. The Ap fragment (0.1 μ g) was also included in the gel as a control for subsequent hybridisation with this probe. The ethidium bromide pattern (left) and the corresponding DNA material that hybridises with the probe (right) are shown in pairs. Tracks (a) and (b), Ap fragment (control); (c) and (d), *oriJ*-Ap $\times$ *EcoRI*; (e) and (f), *oriJ*-Ap $\times$ *HindIII*; (g) and (h), *oriJ*-Ap $\times$ *EcoRI* $\times$ *HindIII*. Molecular weights of the fragments are given in megadaltons and were calculated using λ cf857 DNA $\times$ *EcoRI* fragments as standard. **B**, Physical map of the *oriJ*-Ap plasmid based on data in (A). Broad curve, Ap fragment; thin curve, chromosomal material; arrows, *HindIII* restriction target. The two *EcoRI* targets are at the two junctions of the Ap fragment and the chromosomal DNA. **C**, Hybridisation analysis of *E. coli* DNA with *oriJ*-Ap. *oriJ*-Ap (0.5 μ g) was used as a ^{32} P-labelled probe to test for hybridisation with DNA from *E. coli* strains C600 and KL228 which had been digested with *EcoRI* and/or *HindIII* enzymes. All operations were basically as described in Fig. 1 except that the electrophoresis was run for 12 h at 20 V. Tracks (a), (b) and (c) show respectively hybridisation found against C600 DNA digested with *EcoRI*, *HindIII*, and both enzymes. Tracks (d) and (e) show hybridisation found in KL228 treated with *EcoRI* and *HindIII* respectively. Molecular weights of the hybridisation bands are given in megadaltons and were calculated as before. **D**, Two alternative *HindIII* maps (a and b) of the homologous region to *oriJ* in the *E. coli* chromosome based on data in (C) assuming that the orders of the fragments in the chromosome and the *oriJ* probe are identical (thick line).

be important. In particular it seemed possible that the use of an Hfr strain as recipient might favour the isolation of *oriC* plasmids because in such strains the predicted interference of such a plasmid with chromosome replication as a consequence of control of copy number²² might be alleviated because chromosome replication could be driven by the integrated F factor^{23,24}. We also noticed that on rich medium there was selection against colonies containing the *oriJ*-Ap plasmid. We therefore prepared a new ligation mixture of *EcoRI* digested C600 DNA (1.5 μ g) and Ap DNA (1.5 μ g), transformed the Hfr LC248 (ref. 2) with it and selected ampicillin-resistant colonies on LB medium containing 20 μ g ml⁻¹ ampicillin as indicated by Yasuda and Hirota². Five colicin E1 sensitive clones which segregated ampicillin sensitive cells were found among 300 ampicillin resistant colonies. Plasmid DNA (pLG6) isolated from one of these colonies chosen at random was analysed in the manner described for *oriJ*-Ap. It was found to be indistinguishable from *oriC* on the basis of its distribution of *HindIII* and *EcoRI* sites and homologous DNA fragments of similar size were found in C600 DNA. In addition, the sizes of the fragments which hybridise with *oriC* are those predicted if the *HindIII* sites adjacent to the 5.9-megadalton *oriC* fragment are at the locations found by Marsh and Worcel³. Thus we conclude that pLG6 is probably identical to *oriC*. This conclusion is confirmed by the data shown in Fig. 3 which also demonstrate that *oriC* and *oriJ* do not cross-hybridise with each other. In this experiment, DNA from the bacteriophage λ asn 132 (ref. 25), a hybrid λ which contains a segment of chromosomal DNA including an origin of replication within a 5.9-megadalton *EcoRI* fragment^{25,26}, was cleaved with *EcoRI* and the *EcoRI* fragments tested for homology with pLG6 by hybridisation. An *EcoRI* digest of *oriJ*-Ap was included in the same experiment. It can be seen that pLG6 hybridises with the 5.9-megadalton fragment of λ asn 132 (Fig. 3a, b), thus confirming that pLG6 is homologous with the *oriC* region as defined by others^{2,3,25,26}. In addition, there is no hybridisation between pLG6 DNA and the 8.3-megadalton fragment of *oriJ* indicating that the two origin plasmids share no significant homology (Fig. 3c, d).

Fig. 3 Hybridisation analysis of λ asn 132 and *oriJ*-Ap with *oriC*-Ap. *oriC*-Ap was labelled with ^{32} P and used as a probe to test for hybridisation with λ asn 132 DNA (0.3 μ g) digested with *EcoRI* and *oriJ*-Ap DNA (0.2 μ g) digested with *EcoRI*, basically as described in Fig. 1. λ asn 132 was isolated from strain CM848 (*E. coli* K12 F⁺, *thi. asn* (λ asn 132, λ cf857 S7): source K. von Meyenburg) using published methods³⁰. Tracks (b) to (d) show, in pairs, the ethidium bromide pattern (left) and the corresponding hybridisation picture (right): (a) and (b) λ asn 132 DNA $\times$ *EcoRI* (c) and (d) *oriJ*-Ap $\times$ *EcoRI*. The hybridisation pattern found in the *oriC* region was obtained after 3 h exposure of the autoradiograph and the one found in the Ap region after 60 h exposure. Molecular weights of the λ asn 132 *EcoRI* fragments (megadaltons): 8.2, 6.0, 4.7, 3.7 (3.5), 2.5, 2.0, 1.2.



In relation to the location and functions of the segment of chromosomal DNA which gives rise to *oriJ* plasmids, it is pertinent to note that several authors^{27,28} have postulated the existence of a cryptic prophage integrated in the *sbcA-rac* region of the *E. coli* chromosome and that interestingly the strain that we used as a host for *oriJ*-Ap lacks this prophage. It is noteworthy to mention in this respect that we found a drastic reduction in the transformation frequency with *oriJ*-Ap in some other strains of *E. coli* K12. A full structural analysis of the *sbcA-rac* region in C600 and AB2463 strains and a study of the replication of *oriJ*-Ap in these hosts is in progress.

It is not yet clear to what extent the efficiency of isolation of *oriC* plasmids is influenced by the use of an Hfr recipient as opposed to an F⁻. We have observed that the Hfr recipient LC248 and the F⁻ recipient AB2463 are transformed with similar efficiency by *oriC*-Ap with selection on either M9 or LB media but that the *oriC* plasmid caused a much more severe inhibition of growth of the F⁻ strain than of the Hfr particularly on M9 medium. The basis of these differences in growth rate are being analysed in more detail and in particular the hypothesis that chromosome replication is driven by the inserted F plasmid in Hfr *oriC* strains is being tested directly.

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Stereochemistry of phosphoryl group transfer using a chiral [¹⁶O, ¹⁷O, ¹⁸O] stereochemical course of alkaline phosphatase

THE recent synthesis and stereochemical analysis of a phosphate monoester chiral at phosphorus by virtue only of the three stable isotopes of oxygen^{1,2} opens the way to the determination of the stereochemical course of both chemical and enzymatic reactions that involve the transfer of a phosphoryl group. We report here the first determination of the stereochemical consequence at phosphorus of an intermolecular phosphoryl transfer reaction. In studying the reaction catalysed by alkaline phosphatase we had two aims: first, to discover the stereochemical course of this well-studied transfer, and second to devise a general method by which the configuration of any chiral phosphate monoester can be determined.

The alkaline phosphatase from *Escherichia coli* is a nonspecific phosphate monoesterase³, and there is good evidence that the readily-formed phosphoryl enzyme is a reaction intermediate in the mechanistic pathway normally followed by this enzyme³. As with many other enzymes that catalyse hydrolytic reactions, alkaline phosphatase readily catalyses the transfer of phosphoryl groups to acceptors other than water, such as alcohols⁴. As our method for determining the absolute configuration of a chiral [¹⁶O, ¹⁷O, ¹⁸O]phosphoryl group requires its attachment to one enantiomer of propane-1,2-diol, this compound was used as the acceptor in the present experiments.

Phenyl[(*R*)-¹⁶O, ¹⁷O, ¹⁸O]phosphate was prepared as the crystalline dicyclohexylammonium salt by the method outlined in Fig. 1, which closely parallels that used earlier to prepare [1(*R*)-¹⁶O, ¹⁷O, ¹⁸O]phospho-(*S*)-propane-1,2-diol¹. The disodium salt of chiral phenyl phosphate (270 mg) was dissolved in water (4.0 ml) containing KHCO₃ (1.2 mmol), K₂CO₃ (1.2 mmol), Zn(OAc)₂ (30 nmol), and Mg(OAc)₂ (3 μmol). (*S*)-Propane-1,2-diol (4.0 ml) was added, and the reaction initiated with alkaline phosphatase (10 units *E. coli* enzyme, Sigma). When 49% of the phenyl phosphate had been solvolysed, the reaction was stopped by the addition of Dowex 50 (H⁺ form). The mixture of 1- and 2-phosphopropane-1,2-diols was isolated by ion-exchange chromatography on DEAE-cellulose and on Dowex 1 in 30% yield (based on the phenyl phosphate consumed). This material comprised the 1- and 2-isomers in 9:1 ratio, from which the 1-isomer of phosphopropane-1,2-diol was obtained (containing <3% of the 2-isomer) by crystallisation of the dicyclohexylammonium salt from acetone-water at -20 °C. The isotopic composition of the peripheral phosphate oxygens was: ¹⁶O, 43.7%; ¹⁷O, 15.3%; and ¹⁸O, 41%. These percentages were identical (to within 1%) with the isotope ratios for the peripheral oxygens of the phenyl phosphate starting material.

The absolute configuration of the product 1- [¹⁶O, ¹⁷O, ¹⁸O]phosphopropane-1,2-diol was then determined by the sequence described earlier¹ (this involves ring closure, methylation of an exocyclic oxygen, separation of the resulting diastereoisomeric triesters, methanolytic ring opening, and linked-scan metastable ion mass spectrometry). The configuration at phosphorus in the product was found to be *R* (92% ± 6%), showing that the phosphoryl group has been transferred with overall retention of configuration at phosphorus.

Of all enzymes that involve the transfer of a phosphoryl group (including phosphatases, phosphokinases and phosphomutases), the alkaline phosphatase from *E. coli* is one of the most thoroughly investigated. The evidence that the isolable phosphoryl enzyme is indeed an intermediate on the normal reaction pathway is good³, and the process is evidently of the 'double-displacement' class⁵. It is therefore gratifying to find

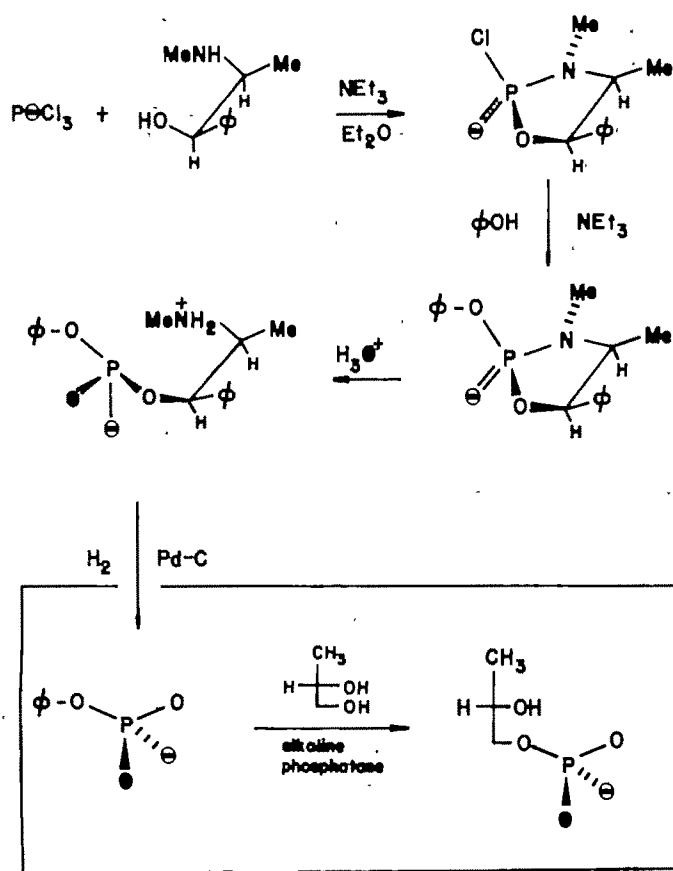


Fig. 1 Synthesis of phenyl[(R)- ^{16}O , ^{17}O , ^{18}O]phosphate and enzyme-catalysed transfer of the chiral phosphoryl group to (S)-propane-1,2-diol.

that the stereochemical consequence of this reaction is indeed retention. While we cannot yet define the stereochemical course of each of the two single displacement steps (the formation of the phosphoryl-enzyme and its collapse), the probability that the phosphorylation is effectively (even if not precisely) the microscopic reverse of the dephosphorylation, makes overall retention the anticipated result. Further, this work in principle allows the absolute configuration of any chiral [^{16}O , ^{17}O , ^{18}O]phosphate monoester to be determined, facilitating the definition of the stereochemical course of phosphoryl transfer reactions that are mechanistically ambiguous.

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Nanosecond transient Raman spectra of photolysed carboxyhaemoglobin

CONFORMATIONAL CHANGES accompanying the photolysis of carboxyhaemoglobin (CO-Hb) have been the subject of much recent study. It is known from time resolved absorption studies that the process is not simple, comprising various steps on time scales ranging from picoseconds to milliseconds¹⁻⁶. Removal of the CO molecule is complete within picoseconds⁵, but the existence of an unknown transient with a 100 ns lifetime has been demonstrated¹. No conclusion has been possible as to the time required for the reorganisation of the porphyrin core. In principle, resonance Raman scattering offers a more detailed source of conformational information. From steady state Raman spectra, the normal modes have been identified^{7,8}. A clear empirical correlation has been found⁹ between the position of the resonantly enhanced anomalously polarised line in the region 1,550-1,590 cm^{-1} , and the porphyrin core size. Here we report the first nanosecond time-resolved spectra of CO-Hb. From our results it is clear that the main reorganisation of the porphyrin core occurs within 5 ns of photolysis.

Human haemoglobin was prepared according to the standard methods. It was reduced with sodium dithionite and then isolated in 0.01 M phosphate buffer (pH 7) by Sephadex chromatography. This stock solution was stored as CO-Hb under an oxygen-free atmosphere. The samples of CO-Hb diluted from this stock solution were sealed under ~1 atm CO. Solutions of deoxy-Hb were sealed under an N_2 atmosphere after the samples had been rendered free of both O_2 and CO in photolytic conditions. The concentration of the samples, measured using optical absorption in the region 5,300-5,700 Å, was typically 0.1-0.3 mM haem.

The spectra were recorded on an optical multichannel analyser of our own design, which has been previously described¹⁰. The time resolution of this apparatus has been increased by more than four orders of magnitude by the use of the pulsed Nd:YAG laser described below. A spectral region up to 200 cm^{-1} wide may be observed, thereby eliminating problems associated with shot-to-shot variation of laser power when pulsed lasers are used. When required, it is possible to gate the input slit using a Kerr cell with a switching time of 3 ns. The time resolution is then improved to ~5 ns. In the spectra reported here, the input slit was set at 250 μm , corresponding to a spectral resolution (FWHM) of 6 cm^{-1} . In all cases the spectrometer was calibrated as to absolute position by measurements on the 1,588 cm^{-1} line of benzene to eliminate backlash effects in the gratings.

Two excitation sources were used. The pulsed spectra were excited by a Nd:YAG laser, frequency-doubled to provide nominal 20 mJ pulses of 5,320 Å radiation with a duration of 10 ns, and a pulse repetition rate of 10 ps^{-1} . In order to obtain cw spectra in similar resonance conditions, a Kr⁺ laser was used, operating at 5,308 Å, at a power of 10-20 mW. The cw beam was sharply focused (~100 μm), while, to avoid damage to the sample container, it was necessary to defocus the YAG pulse to provide a beam diameter of ~750 μm . The resulting peak intensity of 400 MW cm^{-2} is below threshold for any stimulated processes in this system. Both sets of spectra were taken in a rotating sample cell cooled externally by flowing cold nitrogen gas. For the pulsed spectra, these conditions yield a lifetime for the CO-Hb in the laser beam of about 0.3 ns, while for the continuous wave spectra it is estimated to be about 1 ms. Since the latter time is much longer than the time for the rotating cell to turn through the sample volume (~150 μs), the cw spectra represent largely unphotolysed material. In the pulsed case, on the other hand, the first 5% of the pulse was sufficient to photolyse the entire sample. Hence the pulsed spectra represent

mainly photolysed material within 10 ns after photolysis. Since the rotation time of the cell (100 ms) was much larger than the 10 ms recombination time at 1 atmosphere of CO, each pulse was presented with essentially a fresh sample for study. Each spectrum reported here represents the accumulation of 1,600 or more laser pulses.

The essential results are demonstrated by the spectra shown in Fig. 1. The cw spectrum (Fig. 1a) shows the strong anomalously polarised 1,585 cm^{-1} peak characteristic of low spin haem¹¹. The pulsed spectra of CO-Hb (Fig. 1b) obtained with the entire 10 ns pulse, exhibit the disappearance of this line, and the appearance of another strong feature in the region 1,545–1,555 cm^{-1} . This feature seems to consist of two unresolved lines, similar to the spectrum of deoxy-Hb taken in the same conditions. Spectra on a sample of oxy-Hb showed little evidence of photolysis, as expected. The anomalously depolarised component of the doublet (B) is displayed more accurately by subtraction of the weighted polarised spectrum (VV) from the depolarised (VH), using the fact that the depolarisation ratio for the lower member of the doublet is 0.75, by symmetry. Typical results are shown in Fig. 1c. The observed peak positions are tabulated in Table 1. The line B has been found to be correlated

Table 1 Observed line positions (cm^{-1})

Sample	A (dp)	B (ap)	C (dp)
CO-Hb (cw)		1,585 $\pm$ 2	1,634 $\pm$ 2
CO-Hb (pulsed)	1,544 $\pm$ 1	1,554 $\pm$ 1	1,601 $\pm$ 1
Deoxy-Hb (pulsed)	1,547 $\pm$ 1	1,556 $\pm$ 1	1,606.5 $\pm$ 1
Oxy-Hb (pulsed)		1,587 $\pm$ 3	1,640 $\pm$ 3

with porphyrato core size. Hence, the spectra shown demonstrate that the major change in core size occurs in less than 10 ns after photolysis. By the use of the Kerr cell it was possible to observe separately the scattering from the first and second

halves of the 10 ns laser pulse. These spectra, although noisier than those shown in Fig. 1, were essentially the same, clearly showing that the main change in the porphyrato core size occurs within 5 ns of photolysis. Hence, this reorganisation is completed well before the 100 ns lifetime of the transient observed in ref. 1.

The better quality of the spectra obtained with the full 10 ns pulse, shown in Fig. 1, allows the further conclusion that definite (small) shifts of all three lines are observed between the pulsed spectra of CO-Hb and deoxy-Hb. In the case of CO-Hb, the process is initiated with the haem in the *R* configuration, while the deoxy-Hb it is in *T*. The *R*–*T* transition occurs on a much longer time scale¹. Hence, the small differences observed may be associated with the changes in haem geometry during the *R*–*T* transition. However, we should note the possible existence of a long-lived electronically excited state⁶ in either case (or both) which might itself cause perturbations of the Raman frequencies. Further, the correlation observed between core size and Raman frequency⁹ shows a scatter of 4–5 cm^{-1} . Therefore, the shifts we observe, although apparently indicative of a change of 0.02 Å in the core size, may instead be the result of the protein reorganisation. Therefore, we cannot state conclusively on the basis of our spectra that a change in haem geometry is associated with the *R*–*T* transition.

As has been noted by others¹², the observation that the core size changes after photolysis does not necessarily imply a movement of the Fe ion out of the plane. Possible distortions of the haem from a planar geometry may also be involved, thus complicating this portion of the structural analysis. The use of different probe frequencies to achieve resonance with various electronic transitions, may eventually be of utility in resolving this point.

To summarise, we have shown that within 5 ns of photolysis, the core size in CO-Hb assumes a value closely approximating that in deoxy-Hb in the steady state. The observed Raman peaks for the transient species are slightly shifted from those of *T* deoxy-Hb. It seems possible that this corresponds to a transient core size about 0.02 Å larger than that of *T* deoxy-Hb. However, it is not possible to relate these differences more definitely to structural modifications. Moreover, we cannot yet rule out the possibility that additional changes, perhaps non-monotonic ones, in the spectrum may occur over a longer time scale, yet before the *R*–*T* transition. To investigate the possibility of the occurrence of such movement, we plan to use a double-pulse technique to extend this investigation to a wider range of times. Further, a transient Raman investigation of the entire *R*–*T* transition is clearly called for, making use of the variety of resonances available to probe other vibrational excitations.

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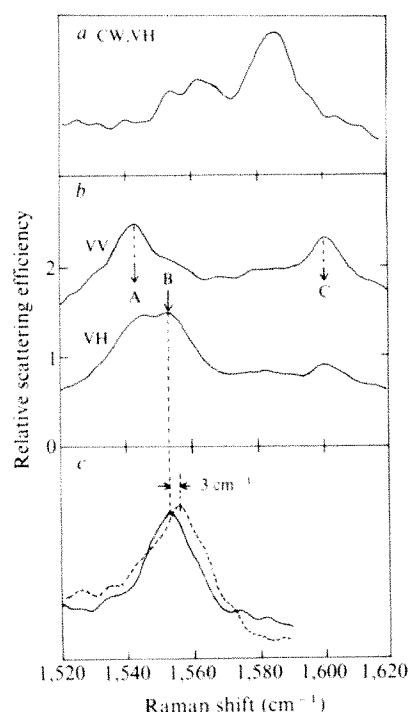


Fig. 1 a, Perpendicular polarised cw spectrum of CO-Hb, obtained with 5,308 Å excitation, at low power. b, Parallel (VV) and perpendicular (VH) polarised transient spectra of photolysed CO-Hb. c, The solid line represents the anomalously polarised component of the transient, obtained by subtraction of the VV spectrum in (b), multiplied by 0.75, from the VH spectrum. The dashed line represents the result of the same analysis for the pulsed spectra of deoxy-Hb.

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matters arising

On melting icebergs

HUPPERT AND TURNER'S laboratory studies¹ of ice melt in warm, salt-stratified water, which demonstrate a system of tilted convection layers of melt mixture interleaved with ambient fluid, are useful in focusing research upon the complex phenomenon of iceberg melt but can not be extrapolated so directly to *in situ* Antarctic iceberg melting as the authors have done, for several reasons: (1) density profile data corresponding to the *T, S* profiles of the Weddell Sea station² shown in Huppert and Turner's article are the depth ranges (and the corresponding density gradients)³: 30–50 m ($1.6 \times 10^{-7} \text{ g cm}^{-3} \text{ cm}^{-1}$); 50–100 m ($1.5 \times 10^{-8} \text{ g cm}^{-3} \text{ cm}^{-1}$); 100–300 m ($2 \times 10^{-9} \text{ g cm}^{-3} \text{ cm}^{-1}$); laboratory study¹ ($1.6 \times 10^{-3} \text{ g cm}^{-3} \text{ cm}^{-1}$); as the mean density gradient has a major influence on the vertical scales of free convection it is difficult to see how laboratory results can describe the oceanic iceberg case where this parameter is from 10,000 to 1,000,000 times smaller. In fact, the density difference over the typical 1 cm vertical scale of a single layer in the laboratory study is six times larger than the total difference in density from 30–150 m of the Central Weddell Sea station. (2) At the 23 °C temperature of Huppert and Turner's salt-stratified laboratory experiment, the thermal expansion coefficient is $0.17 \times 10^{-3} \text{ g cm}^{-3} \text{ }^{\circ}\text{C}^{-1}$; in typical Antarctic waters it is 0.05×10^{-3} , less than one-third as effective in controlling density changes relative to the saline contraction factor which is much the same in both cases (0.81×10^{-3} as opposed to $0.76 \times 10^{-3} \text{ g cm}^{-3} \text{ p.p.t.}^{-1}$, respectively). Furthermore, ambient water temperature in Antarctic water is only about 2 °C above freezing point, an order of magnitude different from their laboratory case. The overall thermal effect on density is then between one and two orders magnitude less in the Antarctic situation.

(3) Antarctic icebergs contain some 7% by volume of compressed air^{4,5}. Effects of air bubble release with melt water at depth are unknown but must be important in modifying vertical convection and horizontal heat diffusion across the boundary layer. A characteristic hissing sound has been reported near icebergs (Seelye Martin, personal communication).

(4) The tilted convection layers observed by Huppert and Turner occur at vertical increments of 1 cm and $2.5 \times 10^{-3} \text{ g cm}^{-3}$ density difference. It required the available heat in 3.5 g ambient fluid to melt 1 g of ice; complete mixing would

result in a mixture of density about $5.6 \times 10^{-3} \text{ g cm}^{-3}$ less than that of the ambient fluid supplying the heat. The characteristic vertical scale of layers ought to have been >2 cm; it was not, which indicates that some melt heat was supplied by means other than turbulent diffusion, such as, double-diffusion. In the Antarctic iceberg case, for reasons (1–3), turbulent diffusion should dominate over other transfer mechanisms. While field measurements are lacking, data reported by Matthews and Quinlan⁶ for Muir Inlet, Alaska, showed that late winter modification of the bay water by glacier melt followed a 21:1 ratio. The ambient fluid at 3 °C and 31‰ salinity supplied about 96 cal g^{-1} of icemelt and part of this must have been needed to raise the ice temperature to melt point. Thus from the only available field data it seems that ice melt entrains ambient fluid with a ratio governed at a minimum by the heat available through complete mixing with ambient fluid, and not less as in the laboratory results¹.

(5) Step structure observed in Central Weddell Sea profiles cannot be described as a plausible extension of the laboratory results. The thickest layer in the given profile extends from 100 to 200 m with a total density difference of $2 \times 10^{-5} \text{ g cm}^{-3}$; a 1,250:1 mixing ratio is required to alter 1 g of melt water to ambient fluid density during its rise through this vertical distance, and the 2,250 cal of available heat in the entrained fluid far exceeds that needed for melting.

A plausible extrapolation of the laboratory results of Huppert and Turner to the melting iceberg would state that the berg generates one such convective layer. A single cell convection was the basis of my earlier study³ on upwelling by icebergs. Even so, the existence of low horizontal temperature and vertical density gradients, compressed air bubbles in glacier ice and reduced thermal expansion effects, and velocity shear associated with berg motion severely limit the role of double-diffusion in iceberg melting (an exception might be the melting process of the bottom surface of the berg).

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Kerogen maturation and petroleum generation

UJJIÉ¹ concluded that a one-point reversal in the C–H–O maturation pathway of kerogens of the Cretaceous of the Douala Basin indicated that a large amount of hydrocarbons was released from the kerogen. Other interpretations are possible. The basic problem is whether the anomaly reflects hydrocarbon release from similar kerogens, or indicates a change in the dominant kerogen type. The latter interpretation is appealing for three reasons.

(1) The anomalous point is not (*h*) which lies directly on the projections of the maturation pathways defined by the shallower points (*a–f*) and the deeper points (*i–n*). The anomalous point is (*g*) which has higher C and H and lower O contents than adjacent points. The kerogen at (*g*) is near peak oil generation². If the original kerogen types at (*f*) and (*g*) were essentially identical, the H content of the kerogen should be decreasing with depth, not increasing³. The increase in H content with depth from (*f*) to (*g*) indicates an increasing hydrocarbon generating capacity with depth, and suggests a corresponding change in original kerogen type.

(2) By themselves, the C–H–O relationships between points (*f, g, h*) could be interpreted as primarily CO₂ generation from (*f*) to (*g*) and hydrocarbon generation from (*g*) to (*h*). This explanation is unlikely. Pyrolysis experiments indicate that CO₂ generation is greatly diminished by the time significant hydrocarbon generation begins⁴, as it has at (*f*). In addition, thermogravimetric analyses of the (*f*) and (*g*) kerogens indicate that (*g*) had a 50% greater weight loss at 500 °C than did (*f*). If (*f*) lost CO₂ and reached the composition of (*g*), the weight loss differential would be in the opposite direction.

(3) Kerogen is extracted organic matter. Its C–H–O contents should be identical at the same stage of thermal alteration whether the generated products remained in the kerogen in the subsurface or whether they were released in the subsurface. By itself, kerogen analysis yields no information on hydrocarbon release.

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UJIIÉ REPLIES—The Cretaceous kerogen of the Douala Basin is the best material available to provide a corroborative evidence of my hypothesis on the reversal in the C-H-O maturation pathway of kerogen, because the Cretaceous is composed of the thick argillaceous sedimentary series in which the original organic matter is identical throughout the whole cores taken between 700 and 4,000 m in depth^{1,2}.

Jones concluded that the anomalous point (g) had a different type kerogen. A series of geochemical studies on the organic matter of the Douala Basin indicates the same kind of organic matter provided in the sample (g) as in the other ones¹⁻³. Tissot *et al.*⁴ pointed out that the whole evolution path of these kerogens is the typical example of their 'type III'.

In order to examine the original kerogen type the maceral components of kerogen should be discussed, instead of the minor difference of hydrogen content and weight loss by pyrolysis as was stated by Jones. The maceral component of each kerogen of the Douala Basin almost consist of more than 80% of amorphous organic cement optically close to vitrinite¹, indicating the homogeneous components throughout the whole cores. Therefore, the C-H-O component path from (g) to (h) should be explained by a large amount of hydrocarbon release, not by the difference of original kerogen type in (g).

Kerogen is extracted organic matter. Its C-H-O content should be related to what kinds of the generated products are released from it, if the original kerogen is identical throughout the strata. The same reversal in the C-H-O maturation pathway as shown from (g) to (h) in the Douala Basin is observed in the Miocene argillaceous sediments of the MITI Borehole in Northern Hokkaido. Examination of hydrocarbon-generating capacity by pyrolysis supports that the relative decrease of carbon content is due to the hydrocarbon release in the MITI Borehole samples (data to be published elsewhere). Therefore, kerogen analysis could give us much information on petroleum generation. To determine whether my hypothesis is applicable to the Douala Basin, the most important work to be done is to decide whether the weight loss by pyrolysis at (g) is due to the generation mainly of hydrocarbon or mainly of CO₂ and/or H₂O.

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Stability problem of Kirkwood Gaps

KIANG noticed¹ the importance of the Kirkwood Gaps (particularly the Hecuba Gap) in contrast to Hilda Group in the asteroidal distribution problem, and presented a solution to this problem using a stability criterion inherited in Hill's equation which, he assumed, clarifies the difference between gaps and groups (less populated against populated regions). His discussion, however, discloses the curious fact that he has omitted the first two terms in RHS of his (linearised) variational equation (6), without any convincing ground. This omission causes a critical deviation from the original problem.

Kiang's discussion starts essentially from the canonical equations of motion with one degree of freedom,

$$\frac{dx}{dt} = F_y \quad \text{and} \quad \frac{dy}{dt} = -F_x \quad (1)$$

where the subscripts refer to the partial derivatives with respect to the variables x and y , respectively. Let $x(t)$ and $y(t)$ be periodic. Then he deduces the variational equations,

$$\left. \begin{aligned} \frac{d\xi}{dt} &= F_{yx}\xi + F_{yy}\eta \\ \frac{d\eta}{dt} &= -F_{xx}\xi - F_{xy}\eta \end{aligned} \right\} \quad (2)$$

where ξ and η are the virtual variations to x and y coordinates, respectively.

He insists that from these variational equations he obtained a second order differential equation of Hill's type. Curiously enough, however, the system of equations (2) can be solved by quadrature as will be seen by equations (9), as it has a (variational) energy integral (see also ref. 2)

$$\mu \equiv F_x\xi + F_y\eta = c_1 = \text{constant} \quad (3)$$

This μ is a variation across the orbit. We also have a variation along the orbit by a form,

$$\nu \equiv F_y\xi - F_x\eta \quad (4)$$

which is perpendicular to μ direction. The differential equation for ν can be obtained by a straightforward manipulation as follows:

$$\frac{d\nu}{dt} = (A\mu + B\nu)/D \quad (5)$$

where

$$\left. \begin{aligned} A &= (F_{xx} - F_{yy})(F_x^2 - F_y^2) + 4F_{xy}F_xF_y \\ B &= -2F_{xy}(F_x^2 - F_y^2) + 2(F_{xx} - F_{yy})F_xF_y \\ D &= F_x^2 + F_y^2 \neq 0 \end{aligned} \right\} \quad (6)$$

On the other hand, we see

$$\frac{dD}{dt} = B \quad (7)$$

Therefore, we can obtain the solution for ν by quadrature, considering that μ is given

by equation (3),

$$\nu = D[c_2 + c_1 \int_0^t D^{-2} A dt] \quad (8)$$

c_2 being another constant. The solutions for ξ and η are readily given by

$$\left. \begin{aligned} \xi &= c_1 F_x D^{-1} + F_y [c_2 + c_1 \int_0^t D^{-2} A dt] \\ \eta &= c_1 F_y D^{-1} - F_x [c_2 + c_1 \int_0^t D^{-2} A dt] \end{aligned} \right\} \quad (9)$$

Note that the solutions by quadrature do not include a new period as in the case of Hill's equation, although the solutions include the mixed secular term, $t \times$ (periodic term) in general. This situation comes from the change of period according as different energy constants (the energy difference being c_1).

Kiang's argument is totally wrong in the point: he only took into account some of the necessary terms without any convincing ground, so that the equation becomes the Hill's type. In reality, the variational equations (2) can be solved by quadrature, because the system has an energy integral. There is no need to introduce a transcendental method, or a new period. In other words, the stability criterion, with which he wanted to clarify the difference between Hecuba and Hilda cases, is not inherent in the variational equations (2) *ab initio*. Thus the problem is still open.

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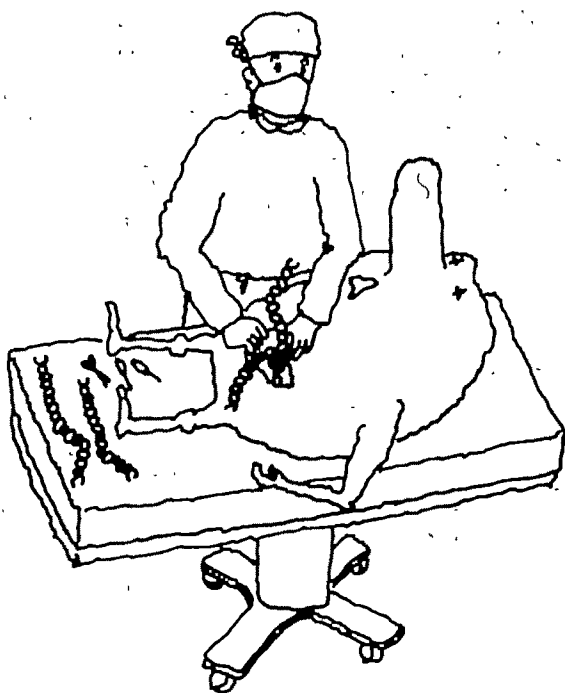
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KIANG REPLIES—Aoki's attitude is the conventional one and is responsible for the lack of success in solving the Kirkwood Gaps problem so far. He apparently believes that because he could write down the solution of the basic equations by the classical method, there is no need to introduce other methods. But the classical method is, in principle, incapable of solving the problem at hand, because it always leads to results (see, for example, Aoki's equations 9) that apply to all resonances equally—it can never explain the Hecuba-Hilda difference. Hence I regard it highly desirable to introduce some new method, equivalent to more or less radical departures from the classical method. Some modification to explain the Hecuba-Hilda difference within the frame of the simplest gravitational model is necessary but as to the form of modification I adopted in my paper, I made it clear that there may well be grounds for improvement. I do not expect any improvement that I could give here to substantially alter the result obtained.

T. KIANG

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Stress Free Anaesthesia

Analgesia and the Suppression of Stress Responses

Edited by C. Wood

September 1978, vii + 89pp., £5.00/\$10.25 0.12.763350.2 (Academic Press) 0.8089.1135.x (Grune & Stratton)

Proceedings of an International Symposium held by the Janssen Research Foundation at Beerse, Belgium on 10 February 1978.

Contents

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General discussion

Chairman's summing up.

*Published jointly by the Royal Society of Medicine,
Academic Press (London) and Grune & Stratton (New York)*

Perimeters of Social Repair

Proceedings of the Fourteenth Annual Symposium of the Eugenics Society, London 1977

edited by W. H. G. Armytage and John Peel

October/November 1978, x + 158pp., £8.80/\$18.25 0.12.062750.7

This volume records the Proceedings of the Fourteenth Annual Symposium of the Eugenics Society, which was held to assess the impact of current programmes of social repair, or, in other words, those acts of professional intervention designed to remedy or alleviate the failure of natural social processes book, which presents the views of a wide variety of acknowledged experts, and avoids, by this diversity, any insistent personal view. It attempts to unravel the myths and dilemmas of social repair as a first step towards establishing better systems of education, health service and social organisation generally.

Proceedings of the Sero Symposium, Volume 17

Obesity in childhood

edited by E. Cacciari, Z. Laron and S. Raiti

September 1978, viii + 248pp., £10.40/\$21.50 0.12.154150.9

Obesity is one of the major health problems today in wealthy developed countries, not only because of the difficulties involved in therapy but also because of the associated problems of changes in endocrine-metabolic functions and cardiovascular disorders. Some of the physiological factors responsible for self maintenance of obesity, for example alteration of the adipose tissue, are thought to originate in early childhood. The purpose of this symposium was to re-examine some recurrent topics such as long-term therapy and the psychological problems of the obese child. Contributions include the experiences and research results of experts from all over the world.

Methods in Microbiology Volume 10

edited by T. Bergan and J. R. Norris

October/November 1978, xvi + 386pp., £17.80/\$36.75 0.12.521510.x

Interests in the factors controlling the spread of pathogenic bacteria in both human and animal population has led, in recent years, to the development of various techniques for the characterisation of isolates from epidemics. These take many forms. Biochemical tests, serological analysis, phage and bacteriocin typing are particularly important. Volumes 10-13 of *Methods in Microbiology* collect together, for the first time, the methods used in identifying all major human and animal pathogenic bacteria of epidemiological importance. The attention to practical detail will enable the methods to be followed in the laboratory, and it is hoped that this will lead to increased uniformity of method around the world.

Photosynthetic Oxygen Evolution

edited by H. Metzner

October 1978, xvi + 532pp., £18.00/\$37.25 0.12.491750.x

The book is a collection of the papers presented during the Symposium "Photosynthetic Oxygen Evolution" held at Tubingen in September, 1977. Photosynthesis specialists, photochemists and electrochemists have contributed towards this multi-disciplinary discussion of the problems of light-induced oxygen evolution. Dealing mainly with the energetics and mechanisms of light-induced charge transfer reactions in green plant cells, the papers include discussions on how plant cells can create a strongly oxidising power; how the oxidised donor regains its missing electrons; and the role of organically bound manganese in the charge transfer. There are also sections on the structure and ontogenetic development of the oxygen producing system of the chloroplasts.

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reviews

Wright's late works

Alan Robertson

Evolution and the Genetics of Populations. Vol. 4: Variability within and among Natural Populations. By Sewall Wright. (University of Chicago: Chicago and London, 1978.) \$46.90; £26.25.

VERDI wrote *Otello* when he was 73 and completed *Falstaff* at the age of 80, though twenty years earlier he had produced some minor works like *Trovatore* and *Rigoletto*. Sewall Wright, though he had contributed extensively to the scientific literature since 1915, published his first book, the first volume in this series of four, in 1968 at the age of 78 and the final two have now appeared in quick succession. The first laid the biometrical and genetic foundations and the second, almost entirely theoretical, extended these to the population level in terms of gene frequencies and concluded with a section on quantitative genetics. The third volume was to discuss the evidence but while the first two were being written so much new evidence became available that it has now become two—the first dealing primarily with evidence from laboratory experimentation and the present one with that from natural populations. The new information came mostly from two new techniques. The first of these, starch gel electrophoresis, has not only produced a great deal of information for the study of population structures (as can be seen from the present contents of journals like *Evolution*) but also answered a previously unanswerable question: What proportion of loci are segregating in wild populations? (the answer is, however, restricted to those loci specifying peptide chains). The second kind of information, from amino acid sequences, can provide a direct genetic measure of the rate of evolutionary substitution at such loci.

Wright's general approach in these final volumes is to analyse specific papers in some detail (sometimes with a re-analysis of his own). For instance, he deals with some work from the early days of genetics like that of Castle on hooded rats (Wright was a junior author of the paper in 1916) and Sumner's work on *Peromyscus*, but also discusses repetitive sequences in DNA and treats extensively the evidence from biochemical polymorphisms in American *Drosophila* populations from Ayala and his colleagues. The volumes

form a valuable reference work and provide a source of critical discussion.

Wright is the last of the three exponents of mathematical studies of evolution made up of himself, Fisher and Haldane, and the final chapters give his summing up of the present situation. The three, working almost independently over about forty years, were very different in temperament and in training. Fisher and Haldane had degrees in mathematics from Cambridge and Oxford, respectively; Wright had a degree in Zoology from a small mid-Western college and continued his experimental work with guinea pigs until he retired from Chicago. Nevertheless, he has always shown extraordinary mathematical insight in solving the problems that he encountered. In fact, a paper of his in 1915 may be considered as foreshadowing the invention of analysis of variance.

He has, I think, always felt that his ideas suffered from a simplistic representation by Fisher and his colleagues. I remember him saying once that "The position got complicated by the appearance of another person in the argument, with the same name as myself but holding completely different views". He felt himself saddled with a naive outlook about the importance of sudden reductions in population size leading to "genetic drift". He was a little surprised to have some British authors scorn "classical random drift" while espousing the "founder principle" which he thought of as a special case of the former. "While it is useful to make such distinctions, it is evident that most of those who have done so have failed to grasp the general principle of effective size in relation to random drift". When Mayr made his criticism of "bean bag genetics" at Cold Spring Harbor in 1959, Haldane felt it necessary to write in reply; Wright did not, he had always taken a more sophisticated view. He returns to the differences in approach on page 464 of the final volume. "It is sometimes supposed, following Mayr (1959), that the early mathematical studies of evolution under Mendelian heredity by Haldane, Fisher and myself were all essentially equivalent, being based on the same simplified biological premises: populations almost homogeneous with respect to an array of

'wildtype' genes except for rare favourable mutations, fixed one at a time, by mass selection, the model referred to by Mayr as 'bean bag' evolution. Actually the processes on which we put major emphasis [Haldane on the basis of major mutations; Fisher on the basis of multiple, largely minor mutations; Wright on the basis of local peak-shifts among interaction systems] were about as different as possible, under the common assumption of Mendelian heredity. Each, however, might be valid for particular characters under particular conditions in species with particular population structures".

His view of the importance of periods of small local population size was not so much that this could of itself lead to fixation in a species but rather that, in an interacting system, such chance changes in gene frequency could allow the population to move, as a consequence of the subsequent selection, from one adaptive peak to another. As with all models involving interactions between many loci, it is not amenable to direct algebraic treatment and Wright's approach has been mainly verbal, dealing numerically with specific examples.

In the past decade the old arguments took on new life in the 'neutralist-selectionist' controversy about the mechanisms of the maintenance of genetic variation within populations (the evidence coming mostly from electrophoresis), and about the rate of gene substitution in protein evolution. Ironically, the neutralist view corresponded somewhat with the more simplistic versions of his views which he had rejected so indignantly in the 1930s. From his discussion on both these points, it is clear that he is in essence a 'selectionist', though he is writing before the recent findings that modifications of electrophoretic technique could increase greatly, at least for some loci in *Drosophila*, the number of alleles known to be segregating in populations.

The fascination of these final volumes comes from the breadth of historical perspective: for the greater part of the development of modern genetics, Wright was really there. □

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Metal sulphides

Mineral Chemistry of Metal Sulfides. By D. J. Vaughan and J. R. Craig. Pp. 493. (Cambridge University Press: Cambridge and London, 1978.) £19.50.

METAL SULPHIDES are not only of concern to geologists in economic, petrological and sedimentological fields but also to metallurgists in many specialisms from the extractive industries to electronics. This book aims to lead all such workers through a variety of experimental and theoretical approaches into a clearer understanding of the range of natural and synthetic sulphides. The authors have made individual contributions to sulphide research and now they have assimilated a vast literature to produce a concise and balanced book.

The text and appendices contain an immense amount of well indexed data with generous references to original work; this will therefore be a satisfying handbook to investigators, but not a manual of experimental techniques. Vaughan and Craig, however, seem to have had the additional objective of teaching the willing reader who might be ignorant of one or other of the particular subjects covered, from crystal chemistry to determinative

methods. In the introduction and elsewhere the underlying theory is introduced straightforwardly and without condescension.

Only four years after P. H. Ribbe's *Short Course Notes* on sulphide mineralogy (Mineralogical Society of America, 1974) it may be asked "why another publication on the same ground?". Although the aims and constraints are here somewhat different, one answer might be that Ribbe showed it could be done. Until that date the variety and complexity of the sulphides defied condensation; their classification was never as straightforward as that of the silicates, and only with the recent deployment of crystal chemistry to the explanation of sulphide structures has a more satisfying rationalisation been glimpsed. Sulphosalts will perhaps be the last to emerge from the jungle but anyone concerned with these not necessarily rarer variants will still have to rely on the reviews of Ribbe's contributors, as Vaughan and Craig dismiss them entirely in order to limit the size of their text.

Naturally this volume is altogether more polished. On the whole, it parallels the 1974 book on sulphide crystal chemistry and classification, with some later work to 1977 included,

and also on chemical bonding, principal phase equilibria of sulphides and associated experimental methods, and current trends and prospects in the investigation of natural sulphides in geology.

Part of the distinctive trend of the book is found in the balancing chapters on electrical and magnetic properties and their applications, spectroscopic studies, classical (that is, including microscopic) determinative methods and sulphide thermochemistry. The chapter on sulphide equilibria in aqueous solutions leads to consideration of conditions in natural hydrothermal fluids and to the thermodynamics of sulphide solubility and deposition.

The final chapter discusses natural sulphides and recognises their differences from the pure and synthetic in their major, minor and trace element contents, solid solution phenomena and S-isotopic variation, the study and significance of all of which are introduced. Appendices contain mineralogical data on 319 sulphides, univariant sulphidation data and invariant points in sulphide mineral systems.

L. G. Love

L. G. Love is Senior Lecturer in Geology at the University of Sheffield, UK.

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Heterogeneous catalysis

Dynamic Heterogeneous Catalysis. By K. Tamaru. Pp. 140. (Academic: London and New York, 1978) £8.50.

IT is difficult to know for whom this volume is intended. Its objective is to emphasise that an understanding of heterogeneous catalysis cannot be achieved by studies of the equilibrium states of solid/gas systems, but must be based on a study not only of the kinetics of catalysed reactions but also of the changes in adsorption on, and chemical properties of, the catalytic sites under working conditions. This thesis is widely accepted and has indeed been the objective of much research, particularly by the author and his coworkers. The complementary view, that one must first understand the nature of clean surfaces and their interaction with simple gases, is, however, implied by a great deal of recent work using modern techniques of surface physics, so that an attempt to strike a balance between the two would be timely.

One therefore expects this to be a research monograph. Instead, one finds a very uneven text including a great deal of elementary physical chemistry (for example, kinetic derivation of the

Langmuir isotherm, Langmuir-Hinshelwood kinetics, and a discussion of homogeneous gas reactions) with which the average research worker in the field will be very familiar. Yet the presentation is not sufficiently balanced to serve as a supplementary undergraduate text. The descriptions of various surface physics techniques—FEM, FIM, LEED, ELS, AES and PES—are very sketchy and hardly seem justified as only scant reference is made to them later in the book: again the research worker in this field will already be very familiar with these techniques.

The book consists of four chapters on: general reaction kinetics; adsorption; the kinetics of heterogeneous reactions; and the applications of spectroscopic techniques to the dynamic treatment of adsorbed species in reaction conditions. Only in the last chapter, in which the examples are drawn largely from the author's own work, does the main theme of the book begin to dominate.

Libraries carrying a comprehensive collection on catalysis will no doubt wish to have this book, but it is unlikely to attract a wide readership.

D. H. Everett

D. H. Everett is Leverhulme Professor of Physical Chemistry at the University of Bristol, UK.

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Understanding living systems

Ordre et Dynamique du Vivant: Chemins de la Biologie Moléculaire. By A. Danchin. Pp. 381. (Editions du Seuil: Paris, 1978.)

THE object of this book, as specified by the author himself, is to emphasise the contribution of our knowledge of molecular phenomena to the understanding of a living system as a whole, including the human brain processes. The book opposes those theories which purport that our understanding of the way a whole organism is working is a specific property of the system and cannot result from the analytical study of each of its components, and concludes that the fundamental properties of a living system are the direct consequences of the properties of its constitutive molecules.

The author chooses several "reduction levels". He first examines the general characteristics of a cell and the modalities of its development. He then considers a second reduction level, the chromosomal one, and includes the rudiments of genetics and indispensable notions on cellular division. In the third and most extensive part, he comes to the fundamental reduction level, the molecular one. After recounting the nature of the interactions between atoms and between molecules, he gives a clear account of the processes of molecular biology—that is, an account of the mechanism of protein synthesis and its regulation—and has succeeded very well in describing the basis of these processes clearly and concisely.

The last part is the most original. It begins with an examination of the nature of viruses and an analysis of the possibility of their synthesis, continues with a study of genetical manipulation experiments already accomplished or to be considered in the future, and ends with an examination of the feasibility of hypotheses concerning the origin of life and the evolution of species. He explains the relationship between phylogenesis and variations in the amino acid sequences of proteins and why he conceives the evolution of species as the development of controls: some fluctuations in the hereditary programme favour the adjustment of the system to external conditions and are therefore integrated in the descendants. There must be a limit, however, to what can be recorded in a genetical programme, and non-hereditary modifications will occur. These individual processes, which the author calls epigenesis, allow adjustment to the external medium. The epigenesis of the nervous network is given as a good example.

This book is aimed to non-specialists. My main regret is that the author's care in explaining clearly the physicochemical mechanisms of the biological processes to non-initiates did not allow him to develop his own ideas more extensively. Nevertheless, this interesting book succeeds well in its stated purpose of showing that the working

of living systems can be explained by the behaviour of their constitutive molecules and their appearance by physicochemical phenomena.

J. Tonnelat

J. Tonnelat is Professor of Physicochemical Biology at the Université de Paris-Sud, Orsay, France.

Regulators of plant growth

Natural Plant Growth Inhibitors and Phytohormones. By V. I. Kefeli. Pp. 278. (Junk: The Hague and Boston, 1978.) Dfl.90; \$44.

THE known and accepted hormonal regulators of plant growth and development comprise the auxins, the gibberellins, the cytokinins, abscisic acid and (perhaps) ethylene. Each of these substances has a clearly defined range of physiological activities, and it is the interplay of these activities which results in the coordination and regulation of the various processes and development. Some plant physiologists, however, believe that this is not enough, asserting that the precise regulation of a process may only be achieved through a balance between stimulatory and inhibitory actions. Given this premise, it is inescapable logic that the auxins, gibberellins and cytokinins, whose actions on cell growth and differentiation are predominantly stimulatory, should be balanced by endogenous, growth-inhibitory substances. As almost any substance, at sufficiently high concentration, will inhibit plant growth, it is obviously very easy to find such inhibitors; it is, on the other hand, and equally obviously, extremely difficult to prove that such growth-inhibitory substances actually act, endogenously, to inhibit growth. Dr Kefeli is a protagonist of the inhibitor camp, and he uses this book in a forceful and single-minded attempt to convince the reader that his view is correct.

The book is based on the author's work carried out at the Timiryazev Institute of Plant Physiology of the USSR Academy of Sciences between 1961 and 1967, and its most valuable contribution lies in providing, for Western scientists, in English, a readable and well documented summary of work which is not easily available from published literature. It is also useful in bringing together diverse information on the synthesis and hypothetical functions of growth-inhibitory substances.

There are, however, many shortcom-

ings. The background literature is only referred to when the conclusions favour the case being made. Also, doctrines which are currently under question in the West (for example, the Cholodny-Went theory of tropisms, the gene expression theory of phytochrome action, and the presumed control of transcription by auxins and gibberellins) are presented as established fact. Critical analysis of data is practically non-existent. This book seeks to make a case, not to present a reasoned and unbiased account. Thus, only the reader himself can judge whether it is successful. Personally I remain unconvinced.

H. Smith

H. Smith is Professor of Plant Physiology at the University of Nottingham, UK.

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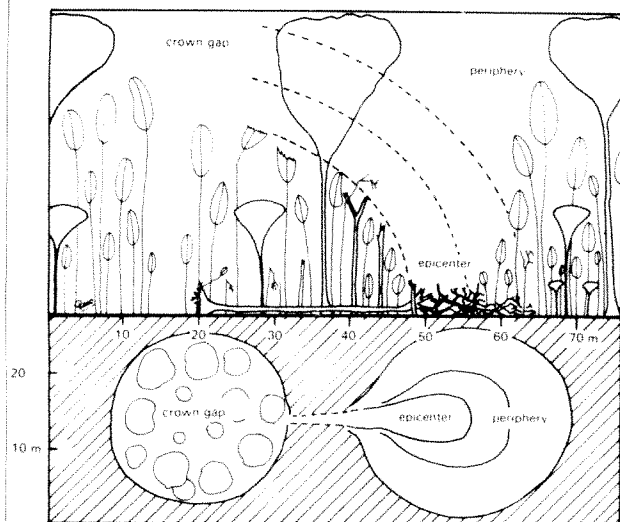
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obituary

Michel Magat

MICHEL MAGAT, professor at the Paris-Sud University of Orsay, died on 6 June 1978. He had been for more than forty years one of the notable physical chemists in Europe.

Born in Kharkov in October 1908, he did his first research in Germany with Ernst Bergman (who was to become one of the organisers of chemical research in Israel) before joining the group of Edmond Bauer at the Sorbonne. There some of his publications made him widely known before he had reached the age of thirty. During the war he was with the Free French Forces until 1945. He then spent a long time at Princeton, adding to his already wide range of interests that of polymeric materials and their behaviour under intense irradiation.

After the war he was among those whom Frédéric Joliot—at the time head of the Centre national de la Recherche scientifique—entrusted with the development of French physico-chemical research. Much of what has been achieved since has been due to him and to his many brilliant pupils. Director of research at the C.N.R.S., he later became assistant professor at the Sorbonne, then full professor. When a Faculty of Science was established at Orsay, he was among the first to go there, and contributed greatly to make it one of the liveliest research centres in France.

He always showed a great interest in his teaching, and in coordinating his lectures with those given on neighbouring subjects. In this too he was a precursor of what has since become commonplace. He was one of those who were not afraid to state that doing good research did not always qualify one to be a teacher.

Radiation effects, polymers, molecular collisions, relaxation phenomena, reaction transients, nearly all aspects of molecular spectroscopy, the structure and behaviour of water, liquids and glasses were among the subjects this encyclopaedic man took up with constant success. His principal concern was fundamental research but he kept a keen sense of realities, and a number of his studies have had useful applications. Graduates came to his laboratory from many countries to benefit from his direction. Throughout the world, his many former students kept with their former 'maitre' the ties of friendship, and often looked for the advice and judgment of his experience and

fertile imagination—the more so as he was a true internationalist, always concerned with improving scientific and cultural exchanges, promoting collaborations and cooperations across frontiers, and also across intellectual and political barriers.

Magat had been president of the Société de Chimie physique, which had recently made him its honorary life president. He was also a vice-president of the Faraday Division of the Chemical Society, and a scientific advisor of the Hahn-Meitner Institute in Berlin. His many travels, his remarkable fluency in many languages, his interest in man and his sense of humour also made him a living repository of the world's folklore and jokes.

Many colleagues and friends mourn his death. Michel Magat achieved much in his research and teaching. He was also fully committed to every cause and principle in which he believed. Age, experience, fame and responsibilities had not dulled in him the sense of justice and duty. He had the friendship and respect of all those who knew him.

C. Troyanowsky

W. D. Chesterman

PROFESSOR W. D. CHESTERMAN, distinguished for work on high speed photography and side-scan sonar, died on 6 July 1978 at the age of 65, after a short illness.

After graduating at Bristol University in 1934 he worked in the research department of the British Thomson-Houston Company. In 1939 his industrial experience proved of value to the Admiralty at the Signal School in Bristol and in 1943 at H.M.S. Vernon. In 1945 he was in charge of the Admiralty Photographic and Instrument Research Laboratory and then moved to the Admiralty Research Laboratory, Teddington.

He was much concerned with the development of techniques involving photography and, in a joint study with industry, made a major contribution to the design of a high speed camera. An underwater study, combining theory and experiment (with short period flash and high speed photography), gave an elegant demonstration of the changing forms of transient, but troublesome, cavitation bubbles.

He became a Fellow of the Institute

of Physics in 1943 and of the Physical Society in 1945. Among his early publications was an authoritative book, on the photographic study of rapid events (1951), which proved of wide interest. His leadership was also recognised by his chairmanship of the International Committee on High Speed Photography (1956–58), and by his Fellowship of the Royal Photographic Society in 1958.

Meanwhile, in 1949 he joined the Admiralty Underwater Weapons Establishment, where his restless enthusiasm and drive stimulated the application of side-scan sonar. He made the first extensive true-plan presentation of sea floor patterns, from an overlapping mosaic of records. The scientific value of the method for showing relief and texture in plan view was illustrated in a joint paper in 1958. In 1959 he was awarded a D.Sc. by Bristol University for his pioneering work in several fields.

In 1960 he made a courageous change by taking the Chair of Physics at Hong Kong. Under his stimulating guidance the department grew in size, became interested in the surrounding seas and attracted visits from other workers. His own research included sonar surveys of nearby sea floors as well as banks of the South China Sea. All was new, but of particular interest was his study of patterned coral floors. He also demonstrated the method in Canadian waters.

In 1966 he became head of the Geophysics Group of Bath University and the Chair of Geophysics was created for him in 1969. He built up the group, attracted funds from outside and encouraged interest in acoustics and the sea. He found a ready demand for side-scan sonar surveys of potential under-sea cable routes and sought to improve search and presentation techniques. Although these aims were commercial the data obtained were scientifically valuable. In 1975 he became head of the Physics School and then became Professor Emeritus just before retirement in 1978. He had been an effective member of University Senate and Council.

His humanity, charm and vivid personality won him many friends and made him easy to work with. These qualities, and his use of hyperbole helped to coax opponents into willing helpers and were valuable aids when handling pomposity. He calmed the nervous students at vivas and had the vital gifts of making young researchers

feel significant and of welding them into an effective team. He had a healthy disrespect for unjustified bureaucracy.

He paid many visits to the mountains and was particularly keen on high level walks. His considerable knowledge and love of music was a pleasure to his friends, an asset to the University Music Society and led to a deep involvement in the management of the Bath Festival. He was largely responsible for the creation of the elegant Crafts Study Centre in Bath.

He leaves his wife Jean and two children Merlyn and Patrick.

A. H. Stride

Vivi Laurent-Täckholm

BIOLOGICAL science in general, and Egyptian botany in particular, has suffered a great loss with the passing of Vivi Laurent-Täckholm, who died in Stockholm on 3 May 1978, at the age of 80.

This grand old lady had been closely associated with the University of Cairo in Giza for over 50 years, some 32 years as a professor of botany in the Herbarium. Vivi Täckholm was recognized and respected not only as the 'dean of Egyptian botany' but appreciated by all who knew her as one of those rare scientist-scholars who is completely selfless. She helped countless students and investigators both in Egypt and abroad by giving freely of her vast experience and deep erudition on all matters Egyptian, motivated only by a sincere hope that a more carefully planned research project could be launched, or if a project was near completion, that a more mature complete interpretation could be achieved.

A brief overview of her life reveals Vivi Täckholm as a very unconventional woman. She was born in Djurs-holm on 7 January 1898. After obtaining her fil. kand. degree in botany (embryology) in 1921 at the Stock-holms Högskolas, she travelled to the U.S.A. where she worked from east coast to west coast at various jobs, including being a servant. She also began at that time her career as a writer, with a two volume work illustrated with her own photographs entitled *Vivi's resa . . .* (Vivi's Journey). This was first published in Stockholm (1923-24) and soon after in German translation (Gotha, 1925-26).

After her return to Sweden, she held a position in the editorial department of the *Nordisk Familjebok* (Nordic Family Book) from 1923-26 (and again in 1931-33). From 1923-25 she also worked as a journalist for various

Stockholm newspapers. She wrote the children's classic *Sagan om Snipp, Snapp, Sorum* (The Saga of Snipp, Snapp, Sorum) during that period (published 1926, last reprinted in 1976.)

In 1926 she married Gunnar Vilhelm Täckholm, a promising botanical investigator who had earned his doctorate at Stockholm in 1922 and had already gained a reputation for his cytological work. Gunnar Täckholm was called as Professor of Botany to the Egyptian University in Cairo (founded in 1908) in September 1925 to found a department of botany in the newly established Faculty of Science. For the next three years, Vivi aided her husband with all those duties associated with running the department (housed in the former harem building of the Za'afaran Palace) and taught the practical botany instruction for students of medicine, pharmacy and dentistry.

During those early years the need for an Egyptian flora was greatly felt, and the plans were laid for the monumental, but still unfinished *Flora of Egypt*. Also a number of major field trips were made and extensive botanical material collected. The Täckholms stayed in Egypt until 1929.

Vivi then spent a year and a half at the Herbarium Boissier in Geneva studying and comparing their specimens with the type specimens of *Flora Orientalis*. She would later acknowledge the importance of this period not only for her understanding of floristics and taxonomy but for the stimulation and encouragement gained from Robert Chodat, the eminent botanist-taxonomist. Vivi Täckholm continued work on identification of Egyptian plants in Berlin, Kew, London, Weimar and Stockholm under scholarships from the Royal Swedish Academy of Sciences. She and her husband continued their collaboration until his death from tuberculosis in 1933.

Vivi Täckholm was persuaded to continue the work her husband and she had started. The Botany Department at Cairo, especially the Herbarium, grew and profited by her association. Under difficult conditions she gradually accomplished wonders. Indeed she called herself 'the world's biggest beggar.' Largely through her labour and efforts, the herbarium grew to be one of the largest and finest in Africa; the same is true of its botanical library.

In 1941, 16 years after its inception, volume I of the *Flora of Egypt* authored by Vivi and Gunnar Täckholm, in collaboration with Mohamed Drar was published as a Cairo University (then Fouad I University) bulletin; volumes II, III and IV appeared in 1950, 1954 and 1966 respectively. These later volumes were co-authored by herself and her Egypt-

tian collaborator and friend Drar. When Drar died in December 1964 at the age of 70, Vivi Täckholm expressed her great concern for the future of the Flora project which she continued to work on until her death.

The *Flora*, not unexpectedly, is an unusual work. It is a labour of love, clearly carried out unhurriedly in the tradition of those great botanical scholars she would emulate. It has been hailed as a model of scholarship and completeness. One special feature is the detailed treatment of the extensive ancient Egyptian tomb flora; another is the attention paid throughout the volumes to agriculturally important plants and economic botany. Also the enormous bibliographies, which provide access to the ancient and modern Arabic literature, are gems. Since the completion of the *Flora* would take many additional years, a version especially aimed at students, was published in 1956 (2nd edition 1973).

In 1952 Stockholms Högskolas conferred upon her an honorary doctorate. In April 1978 she was honored by the Royal Swedish Academy of Sciences with the gold Linné medal for her pioneering research. The Egyptian government bestowed her with high order decorations on her 60th and 70th birthdays. A special Festschrift entitled *50 Years Cairo University Herbarium—80 Years Birthday of Vivi Täckholm* was published in 1977.

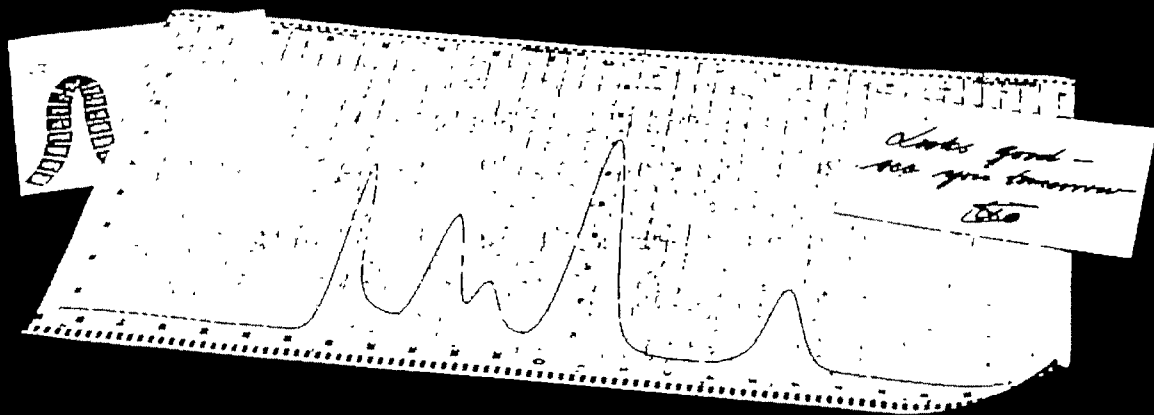
In addition to several shorter, research publications, Vivi Täckholm authored numerous popular and semi-popular books. In these works her supreme knowledge of Egypt as well as her deep love for plants, Egypt, and the Egyptian people is apparent. Indeed she is attributed with saying she 'could live without food, but not without flowers.'

Cairo University has recently decided to name the Herbarium in her honor. But the greatest legacy of Vivi Täckholm is the tradition of love for work and scholarship that she set for her many students and associates. She was also gifted with an exceptional and gentle personality. All who knew her, well or slightly, agree that they are better off, even enriched, by having associated with her. Her smile, bright eyes, fluffy snow white hair, generosity and readiness to help will be remembered warmly by many. Those who had the pleasure of visiting her at her apartment in Orman will also recall her gracious hospitality.

Her closest survivors, a brother, Professor Emeritus Torbern Laurent, her sisters Maj Laurent, textile designer and Gavonne Laurent, several nieces and nephews, and their families, are extended our deepest sympathies.

Abraham D. Krikorian

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The closing date for sending in Papers for the 1979 award is 1st March, 1979.

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The presentation of this year's prize will take place at 10.30 a. m. on 27th October, 1978 in the Adolf-von-Baeyer-Lecture-Hall of the University of Munich, which is located in Meiserstrasse.

Recent scientific and technical books

Physics

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- BASOV, N. G. (edited by). *Coherent Cooperative Phenomena*. Translated from Russian by Donald M. McNeill. (Proceedings (Trudy) of the P. N. Lebedev Physics Institute, Vol. 87.) Pp.viii+154. ISBN-0-306-10945-X. (New York and London: Consultants Bureau, 1978.) \$35.40.
- BENDOW, B., and LINGELER, B. (contributions by). *Electronic Structure of Noble Metals and Polariton-Mediated Light Scattering*. (Springer Tracts in Modern Physics, Vol. 82.) Pp.vi+114. ISBN-3-540-08814-8. (Berlin and New York: Springer-Verlag, 1978.) DM 48; \$24.
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- CAUCHOIS, Y., and SENEAUD, C. *Wavelengths of X-ray Emission Lines and Absorption Edges*. (International Tables of Selected Constants, 18.) Pp.320. ISBN-0-08-022448-2. (Oxford and New York: Pergamon Press, 1978.) £50.
- CHISHOLM, J. R. *Vectors in Three-Dimensional Space*. Pp.xii+293. ISBN-0-520-29289-1. (Cambridge University Press: Cambridge, London, New York and Melbourne, 1978.) £15.00 H/C; £4.95 P/B.
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- HONIGFELD, Gilbert, and HOWARD, Alfreda. *Psychiatric Drugs, a Desk Reference*. Second edition. Pp. xiv + 251. ISBN-0-12-354860-8. (New York and London: Academic Press, a Subsidiary of Harcourt Brace Jovanovich, Publishers, 1978.) \$13.50; £8.75.
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- WEBER, E. H. *The Sense of Touch: De Tactu* translated by H. E. Ross. *Der Tactus* translated by D. J. Murray. Pp. x + 278. ISBN-0-12-740550-X. (London and New York: Academic, 1978. Published for Experimental Psychology Society.) £7.40.

Sociology

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Anthropology

- LEAKEY, Meave G., and LEAKEY, Richard E. (edited by). *Koobi Fora Research Project. Vol. 1: The Fossil Hominids and an Introduction to Their Context, 1968-1974*. (Monograph No. 1 of The International Louis Leakey Memorial Institute for African Prehistory Series.) Pp. xvi + 191. ISBN-0-19-857392-8. (Oxford: Clarendon Press, London: Oxford University Press, 1978.) £15 net.
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History of science

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General

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announcements

Meetings

4–6 December, **Winter Simulation Conference**, Miami (Paul Roth, B250 Technology Building, NBS Washington, D.C. 20234).

5 December, **Automating Building Climatology**, Nottingham (Royal Meteorological Society, James Glashier House, Grenville Place, Bracknell, Berks, UK).

13 December, **Variability in the Frequency of Unusual Weather over approximately the Last Century**, London (Royal Meteorological Society, James Glashier House, Grenville Place, Bracknell, Berks, UK).

13 December, **Computer Networking Symposium**, Gaithersburg (Rob Rosenthal, B212 Technology Building, NBS, Washington, D.C. 20234).

14 December, **Weather Modification**, Manchester (Royal Meteorological Society, James Glashier House, Grenville Place, Bracknell, Berks, UK).

5 January, **Aspects of Current Research in Climatology**, Manchester (Royal Meteorological Society, James Glashier House, Grenville Place, Bracknell, Berks, UK).

9 January 1979, **Radiative Fog—Physical and Forecasting Aspects**, Nottingham (Royal Meteorological Society, James Glashier House, Grenville Place, Bracknell, Berks, UK).

18 January, **Synthetic Alicyclic Chemistry**, London (Stanley Langer, The Chemical Society, Burlington House, London W1, UK).

18 January, **Snails and Hedges—an Ecological and Historical Interpretation**, London (The Linnean Society of London, Burlington House, London W1, UK).

25 January, **Research on African Climatology**, Manchester (Royal Meteorological Society, James Glashier House, Grenville Place, Bracknell, Berks, UK).

26–29 January, **Renal Patho-physiology—Recent Advances**, Florence (Prof. A. Leaf, Harvard Medical School, Massachusetts General Hospital, Boston, Massachusetts 02114).

29 January–24 February, **Approaches to the Molecular Genetics of Oncogenes**

Viruses by DNA Cloning and DNA Injection into Mice Blastocysts, Rome (Prof. Enrico Calef, Centro Acidi Nucleici del CNR, Via G. Romagnosi 18/A, 00196 Rome, Italy).

14 February, **Fracture Mechanisms**, London (The Meetings Secretary, The Institute of Metallurgists, Northway House, Whetstone, London N20, UK).

16 February, **Cellular Recognition Systems**, Edinburgh (The Linnean Society of London, Burlington House, London W1, UK).

20 February, **Climate Research and its Relevance to Human Affairs**, Lincoln (Royal Meteorological Society, James Glashier House, Grenville Place, Bracknell, Berkshire, UK).

21–23 February, **The Value of Meteorological Services**, Victoria (Dr P. Webster, CSIRO, Division of Atmospheric Physics, Aspendale, Victoria, Australia).

3–5 April, **Aspects of Researches into Monocotyledons of Horticultural Importance**, London (The Linnean Society of London, Burlington House, London W1, UK).

4–7 April, **Phase Transformations**, York (The Meetings Secretary, The Institution of Metallurgists, Northway House, London N20, UK).

29–31 May, **International Symposium on Oligozoospermia**, L'Aquila (G. Frajese, Clinica Medica V—Policlinico Umberto I, 00100 Rome, Italy).

2–23 June, **Tropical Marine Ecology**, Pine Clay (Barbara Laumark, University of Connecticut, Box U-56, Storrs Connecticut 06268).

4–8 June, **International Uranium Symposium on the Pine Creek Geosyncline**, Sydney (The Executive Secretary, PO Box 387, Grosvenor St, Sydney, New South Wales 2000, Australia).

4–17 June, **The 1978 CERN School of Physics**, Utrecht (Service de Presse, CERN 1211 Geneva, Switzerland).

8–10 June, **International Symposium on Chemical Toxicology of Food**, Milan (Organizing Secretariat, Centro

Studi Dell' Alimentazione, Nutrition of Italy, Via S. Pietro all'Orto 17, 20121 Milan, Italy).

13–15 June, **Chromium in Nutrition and Metabolism**, Quebec (Dr D. Shapcott, Departement de Pediatrie, Centre Hospitalier Universitaire, Sherbrooke, Quebec, Canada J1H 5N4).

18–22 June, **Occupational Radiation Exposure in Nuclear Fuel Cycle Facilities**, Los Angeles (John H. Kane, Office for Technical Information, Dept of Energy, Washington, D.C. 20545).

19–20 June, **10th International Symposium on Chromatography and Electrophoresis**, Venice (Fondazione Giorgio Cini, Isola di San Giorgi Maggiore, Venice, Italy).

19–22 June, **4th International Symposium on Biochemistry of Exercise**, Brussels (Prof. J. Poortmans, Chimie Physiologique—ISEPK, Université Libre de Bruxelles, 28, Ave P. Héger, B-1050 Brussels, Belgium).

21–22 June, **6th International Symposium on Mass Spectrometry in Biochemistry and Medicine**, Venice (Fondazione Giorgio Cini, Isola di San Giorgi Maggiore, Venice, Italy).

24 June–3 July, **Chemistry of Solid/Liquid Interfaces**, Cavtat (5th Summer Conference Chemistry, Center for Marine Research, "Rudjer Boskovic" Institute, PO Box 1016, YU-41001 Zagreb, Yugoslavia).

1–6 July, **8th International Conference on Atomic Spectroscopy**, Cambridge (Secretariat, PO Box No. 109, Cambridge, UK).

2–4 July, **Insulating Films on Semiconductors**, Durham (Prof. G. G. Roberts, Dept of Applied Physics and Electronics, University of Durham, Science Laboratories, South Rd, Durham, UK).

2–6 July, **Laser 79 Opto-Elektronik**, Munich, (Münchener Messe-und Ausstellungsgesellschaft mbH, Bereich Kongresszentrum, Kongressbüro, Lasre 79 Opto-Elektronik, Postfach 12 10 09, D-8000 München 12, FRG).

6–7 July, **The On-Line Revolution in Information**, Paris (ICSU AB, 17 rue Mirabeau, 75016 Paris, France).

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- The staff member shall be entitled to free sea freight allowance of 400 (four hundred only) kilograms, provided costs of the said freight for the said cargo do not exceed Kuwaiti Dinars 100 only. This is applied at the beginning and end of the contract.
- Gratuity at the end of service shall be one month's basic salary for each year spent in the service of the University.

Application and Curriculum Vitae forms are obtainable from the nearest Kuwait Embassy. Completed application accompanied by three letters of recommendation, together with a non-returned copy of the candidate's publications should be submitted directly to Kuwait University, Department of Administration Affairs, P.O. Box 5969, Kuwait, not later than 31st December, 1978. Short term contract (e.g. sabbatical, visiting, etc.) will also be considered. 7901(A)

COURSES

THE ADVANCED SCHOOL in PROCESS MICROBIOLOGY AND BIOTECHNOLOGY at the UNIVERSITY OF KENT September 16 to 22, 1979

This residential course is intended for graduates in microbiology, biochemistry or engineering working in the multidisciplinary areas embraced by biological processes. Essential features of the course are the "tutorial" system and lectures by eminent visiting speakers.

Course tutors: Dr D. E. F. Harrison, Dr R. C. Righelato, Dr D. Brown, Dr. G. Pace, Dr S. G. Oliver. Visiting Lecturers include: Dr C. Ball, Professor P. H. Clarke, Professor S. J. Pitt, Professor D. W. Tempest, Professor B. Atkinson, Dr D. S. Flynn. Tuition Fee: £150.00 Accommodation (approx.) £90.00.

For further details apply to: Dr S. G. Oliver, University of Kent at Canterbury, Biology Laboratory, Canterbury, Kent, CT2 7NJ.

7947(D)

Agricultural Research Council Institute for Research on Animal Diseases

MOLECULAR BIOLOGIST

A MOLECULAR BIOLOGIST is required to lead a small research team studying the virulence determinants of pathogenic organisms. The binding of the K group of *E. coli* antigen to intestinal receptors has been an especial interest of the team but it is intended to expand work to encompass other related situations.

In addition the person will be expected to advise and assist other project groups.

Qualifications: 1st or Upper 2nd Class Honours Degree in Biochemistry, Molecular Biology or a similar subject and at least four years' relevant postgraduate research experience.

Salary: Senior Scientific Officer scale £5,134 p.a. to £6,898 p.a. or Principal Scientific Officer scale £6,609 p.a. to £8,461 p.a. depending on experience.

Permanent post, with non-contributory pension scheme.

Application forms Secretary, Institute for Research on Animal Diseases, Compton, Nr. Newbury, quoting ref. 274. Closing date for applications October 26, October 1978.

7948(A)

UNIVERSITY OF WOLLONGONG LECTURER IN BIOLOGY (LIMITED TERM) PLANT PHYSIOLOGIST

The Biology Department offers a broadly based first year course followed in second and third years by a coherent series of subjects dealing with functional biology at cellular, physiological and community levels. The appointee will be primarily responsible for a course in plant physiology with emphasis on regulatory mechanisms and environmental interactions, and will also be expected to contribute to other subjects offered by the department. High academic qualifications and significant postdoctoral experience are desired.

Further information may be obtained from Professor A. D. Brown, in the University.

Closing date November 6, 1978

Salary range \$A15,179 to \$A19,940 per annum.

The term of an appointment for a limited term lectureship would be for a period not exceeding four years.

Condition of appointment and application procedure from Association of Commonwealth Universities (Acpa), 36 Gordon Square, London WC1H 0PF. 7921(A)

THE ROYAL VETERINARY COLLEGE University of London

Division of Paraclinical Studies DEPARTMENT OF PATHOLOGY LECTURER IN

VETERINARY PATHOLOGY

Applications are invited from veterinary graduates for the post of Lecturer in Veterinary Pathology. Duties will include participation in teaching the undergraduate and postgraduate courses in pathology at some diagnostic work. The successful applicant will be encouraged to take advantage of the good research opportunities which are available.

Preference will be given to applicants having some relevant experience in postmortem diagnosis, histopathology or similar fields, but consideration will be given to applications from recent graduates or others.

SALARY SCALE (UNDER REVIEW) £4,333 to £8,204 inclusive. London Allowance. Initial salary will be determined according to qualifications and experience. Superannuation under the Universities' scheme.

Removal expenses up to £800 may be reimbursed in approved circumstances.

Application form and further details from the Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU. Telephone 01-387 2898.

CLOSING DATE FOR APPLICATIONS November 6, 1978. 7964(A)

UNIVERSITY OF HONG KONG DEPARTMENT OF ANATOMY LECTURESHIPS/ASSISTANT LECTURESHIPS IN ANATOMY

Following the establishment of Dental School applications are invited for immediate appointments to the two posts of Lecturer/Assistant Lecturer in Anatomy. Applicants should be either medical, dental or science graduates with teaching experience in the subject. Preference will be given to those with experience in teaching dental students. The appointments also carry responsibility for teaching pre-clinical medical students. The appointees will be expected to engage in research work.

Annual salaries (superannuable) are: — Lecturer HK\$63,540 to HK\$4,260 by HK\$4,320 BA HK\$76,320 by HK\$4,320 Assistant Lecturer HK\$106,140 by HK\$4,320 to HK\$59,22 (£1 equals HK\$9.30 approx.) Start salary will depend on qualifications and experience. A medically-qualified appointee who has completed his pre-registration year will be appointed a Lecturer at the minimum point of the scale.

Further particulars and application forms may be obtained from the Association of Commonwealth Universities (Acpa), 36 Gordon Square, London WC1H 0PF, or the Assistant Secretary (Recruitment), University of Hong Kong, Hong Kong.

Closing date for applications November 7, 1978. 7899(A)

STERLING-WINTHROP **STERLING**  **WINTHROP** STERLING-WINTHROP

Chemistry Laboratory Manager

We are currently seeking a manager for the Chemistry Department of our Research and Development Division, and are looking for an enthusiastic individual to co-ordinate and develop current activities.

The Chemistry Department plays an important role in the activities of a Group with wide international commitments. Its functions include the synthesis of novel organic compounds with potential pharmaceutical application, the synthesis of compounds for analytical, reference, patent or metabolic purposes, including radio chemicals, a complete analytical service for the analysis and characterisation of compounds produced within the Department, or supplied by Associated Companies throughout the world, and the assembly of data for the registration of new pharmaceuticals.

The successful candidate will probably be aged 27-32, and have some specific experience of the work carried out in this Department together with several years experience of practical Organic Chemistry. An interest in some of the analytical aspects of Organic Chemistry is also essential.

If you would like to find out more about the position, please contact
Colin Davison,
Sterling Winthrop Research & Development,
Edgefield Avenue, Fawdon,
Newcastle upon Tyne, NE3 3TT
Tel: Newcastle 863931 Extn 19

7963(A)

**Sterling-Winthrop
Research & Development**
A Division of the Sterling-Winthrop Group

STERLING-WINTHROP STERLING-WINTHROP STERLING-WINTHROP STERLING-WINTHROP STERLING-WINTHROP

**UNIVERSITY OF
NEWCASTLE UPON TYNE
DEPARTMENT OF APPLIED
MATHEMATICS**

**Applications are invited for a
POSTDOCTORAL
RESEARCH ASSOCIATE**

to carry out research in the FLUID DYNAMICS of superfluid flow under the direction of Professor P. H. Roberts of the above Department and Dr R. N. Hills of Heriot-Watt University, Edinburgh. The post is tenable up to two years and will commence as soon as possible.

Starting salary will be on national salary scale not exceeding £4,133 per annum according to age, qualification and experience, plus USS contributions.

Further particulars can be obtained from Professor P. H. Roberts, Department of Applied Mathematics, The University, Newcastle upon Tyne, NE1 7RU with whom applications (two copies) together with names of three referees, should be lodged not later than three weeks after publication.

7959(A)



School of
Environmental Sciences

**L II ENVIRONMENTAL
GEOLOGY**

Salary range: £4,101 to £6,558

For integrated foundation year of Environmental Science honours degree course and other degrees, e.g., Economic or Applied Geology.

Experience or interest in integration of Earth Sciences with other disciplines preferred.

Application forms to be returned by Friday, November 17, 1978, can be obtained with further particulars from the Personnel Officer, Plymouth Polytechnic, Drake Circus, Plymouth PL4 8AA.

7949(A)

**QUEEN MARY COLLEGE
University of London**

**Applications are invited for the post of
MOLECULAR GENETICIST**

to undertake research on the genetic manipulation of yeast with special reference to transformation studies. Applicants should have research experience in Microbial Genetics and/or Molecular Biology.

The appointment is sponsored by a major industrial organisation and the selected candidate will be on the permanent payroll of the Company concerned. Following a 2-3-year period of research at Queen Mary College, the successful candidate will be transferred to the Company's own research laboratories.

A competitive salary and non-contributory pension rights in accordance with the Company's own scale will be offered to the successful candidate. Further details will be given at interview.

Applications should be made by October 27 to Professor E. A. Bevan, (N) Professor of Genetics, Queen Mary College, Mile End Road, London E1 4NS.

7963(A)

**THE QUEEN'S COLLEGE
GLASGOW
LECTURER**

with good honours degree in Nutrition or Dietetics or a related science and eligible for State Registration in Dietetics to teach Applied Nutrition and Diet Therapy at Degree and Diploma level.

This is a new appointment. Salary range: £4,056 to £7,167 (bar) to £7,698 per annum. (Full credit will be given for all relevant experience when calculating placement on this scale.)

A senior appointment may be available for an exceptional candidate. Salary range: £7,155 to £7,962 (bar) to £9,042 per annum.

For a member of staff without teaching experience teacher training opportunity would be available.

Assistance with removal expenses. Further details and forms of application may be obtained from The Principal, 1 Park Drive, Glasgow G3 6LP to whom completed forms should be returned not later than Monday October 30, 1978.

7388(A)

MEDICAL RESEARCH COUNCIL

RADIOBIOLOGY UNIT

RESEARCH APPOINTMENTS

Applications are invited for a

SPECIAL APPOINTMENT

and also appointments in other

SCIENTIFIC GRADES

to study effects of ionising radiations on tissues, especially carcinogenesis from radioactive materials in animals.

Applicants should be well qualified in biological sciences, medicine or veterinary medicine and for the more senior posts have several years postgraduate experience. For at least one appointment experience in histopathology will be an advantage. Qualities of leadership and high academic standing are required for the Special Appointment which is the equivalent of University Professor.

Work at the Unit covers a wide range of scientific interests in relation to protection against ionising radiations and radiotherapy.

Salary in accordance with age and experience on M.R.C. Scales (as at October 1, 1978, with negotiations in progress).

Grade II	Grade I	Senior	Special Appointment
£3,883	£6,317	£7,506	£9,443
£6,080	£7,754	£9,263	£12,247

Enquiries or applications with curriculum vitae and names of two referees to Dr J. Vennart, Director, M.R.C. Radiobiology Unit, Harwell, Didcot, Oxon OX11 0RD by November 3, 1978.

7896(A)

**CAPRICORNIA INSTITUTE OF ADVANCED EDUCATION
ROCKHAMPTON QUEENSLAND AUSTRALIA**

Department of Biology

Applications are invited for the position of:

**Senior lecturer/
lecturer in Biology**

Applicants should have at least a good Honours degree preferably in one of the following fields—microbiology, plant physiology, mycology. Suitably qualified applicants in other biological disciplines will be considered.

Industrial and/or teaching experience and a higher degree would be an advantage for appointment as Lecturer and a requirement for appointment as Senior Lecturer.

The successful applicant would be expected to contribute to undergraduate teaching at the degree and associate diploma levels. A significant component of the duties would involve external students and applicants should have a definite interest in this field.

Further details are available from Dr B. Rothwell, Head, Department of Biology (Telex AA48178).

Appointment may be made by means of two or three year contracts or on permanent terms.

Current salary range:

Lecturer \$13,083 to \$18,840 per annum

Senior Lecturer \$20,365 to \$23,737 per annum

Applicants in U.K. or Europe may obtain further information from and make application through the Agent General for Queensland, Queensland House, 392 Strand, London WC2R 0LZ by Friday, 15th November, 1978.

Applications other than from the U.K. or Europe should be addressed to the Registrar, C.I.A.E., M.S. 76, Rockhampton, Qld. 4700, Australia by Friday, 15th November, 1978.

7912(A)

**ROYAL MARSDEN
HOSPITAL
DOWNS ROAD,
SUTTON, SURREY
SCIENTIST
NUCLEAR MEDICINE
DEPARTMENT
£3,840 to £5,253**

Applications are invited from suitably qualified graduates, experienced in radio-isotope techniques, to take charge of in-vitro tests in a broad based Regional Nuclear Medicine Department. Applicants must have an interest in developing new techniques and in being involved in research work.

Salary starting point within range according to experience.

Application forms and job description from Personnel Department, Royal Marsden Hospital, Fulham Road, London SW3 (Tel: 01-352 8171, Ext. 446). Closing date October 23, 1978.

7902(A)

THE UNIVERSITY of Manchester, Department of Psychiatry. A Technician (Grade 4) is required for full-time duties in the Department of Psychiatry, Stopford Building (New Medical School). The successful applicant will work with a small research team studying the effects of drugs on the central nervous system. Duties will include assistance with electro-physiological experiments, photography and general laboratory maintenance. Some knowledge of electronics is an advantage.

The applicant should possess H.N.D. or H.N.C. (or equivalent qualifications) in appropriate subjects and have had at least six years' relevant laboratory experience.

Salary range £2,935 to £3,402 per annum.

Applications, together with a curriculum vitae and the names and addresses of two referees, should be sent to Mrs G. Scott, Department of Psychiatry, University Hospital of South Manchester, West Didsbury, Manchester M20 8LR. Closing date for applications: Friday, October 20, 1978.

7931(A)

**TECHNICIAN
GRADE 2B**

required for the Anatomy Department. The work will involve experimental investigation of the nervous system and/or experimental embryology. Applicants should preferably have experience of histological techniques and laboratory procedures in relation to such studies, but those with more general experience will be considered. Applicants should possess an O.N.C. or equivalent qualification. Salary on scale £2,994 per annum rising on scale to £3,345 per annum inclusive. Four weeks annual holiday. Contributory pension scheme. Apply in writing with full details to: The Head Clerk, Ref 204012/N, King's College London, Strand WC2R 2LS.

7922(A)

**MCGILL UNIVERSITY
DEPARTMENT OF
BIOLOGY
POSTDOCTORAL FELLOW**

required to work in a project on the interaction of the killer toxin protein with cell surface receptors in yeast. Experience in protein chemistry and/or yeast genetics an advantage. The appointment is for one year initially and may be renewed for a further two years. Initial salary \$12,100 per annum.

Applications and two letters of recommendation should be sent to Dr H. Bussey, Department of Biology, McGill University, 1205 McGill Avenue, Montreal, Quebec, Canada H3A 1B1.

7908(A)



**INVERESK RESEARCH
INTERNATIONAL**

CYTOGENETICS

The Division of Reproductive Toxicology at Inveresk Research International is undertaking a phase of major expansion, with emphasis on dominant lethal, sperm abnormality and mutagenic assessment. Significant career opportunities exist for the following staff with qualifications and/or experience in relevant areas:

Senior Cytogeneticist: To head up the activity and provide scientific and management input (ref. 2612).

Assistant Cytogeneticists (3): To assist mammalian cells treated *in vivo* and *in vitro* for chromosomal damage (ref. 2613).

Mammalian Mutation Studies Assistants (2): To perform dominant lethal and sperm abnormality studies in rodents (ref. 2614).

IRI is located on the outskirts of Edinburgh within easy reach of the city and the surrounding countryside. The above posts carry a wide range of staff benefit schemes and relocation assistance is provided if required. Application forms, quoting the appropriate reference, are available from Miss J. M. Dunbar, Inveresk Research International, Inveresk Gate, Musselburgh EH21 7UB.

7943(A)

**UNIVERSITY OF
THE WITWATERSRAND,
JOHANNESBURG
DEPARTMENT OF GEOLOGY
LECTURESHP/SENIOR
LECTURESHP IN GEOLOGY**

Applicants should have substantial field and research experience, with emphasis on modern petrogenetic techniques particularly as applied to mineralised terrains. Although it is not a prerequisite for appointment, if the successful applicant has suitable technical and analytical experience in the field of electron probe microanalysis, he will have the opportunity to supervise the operation of the new computerised ARL-SEMQ electron microprobe.

The starting salary will be determined according to qualifications and experience and will be within the range Lecturer, R7,275 to R10,600 per annum, Senior Lecturer, R9,770 to R12,990 per annum. (£1=R170 approx.)

The University's policy is not to discriminate in the appointment of staff or the selection of students on the grounds of sex, race, religion or colour.

Further particulars relating to this practice and to the above post are included in an information sheet obtainable from the London Representative, University of Witwatersrand, Chichester House, 278 High Holborn, London WC1V 7HE, or from the Registrar, University of the Witwatersrand, Jan Smuts Avenue, Johannesburg, South Africa, with whom applications (together with the names of three referees) should be lodged by October 23, 1978.

7962(A)

**UNIVERSITY OF
TASMANIA
LECTURESHP/SENIOR
LECTURESHP IN
PATHOLOGY**

Applications are invited for 14 positions within the following scale: Senior Lecturer \$A20,365 2 by \$A675 to 3 by \$A674 \$A23,737; Lecturer \$A15,179 to 1 \$A681 to 6 by \$A680 to \$A19,940. An appointment will be offered from the beginning of 1979 within one of these grades, according to qualifications and experience. An additional loading is paid to medical staff with clinical responsibility. For this appointment, it is expected that the load would be \$A5,000 per annum.

Applicants must hold a medical qualification registrable in Tasmania and should have had experience of the practice of pathology. The successful appointee will be expected to contribute to the Pathology service of the Royal Hobart Hospital, where he will be given an appropriate appointment.

Intending applicants are invited to write to Professor R. A. Rodd, Department of Pathology, in the University, for any further information they may require.

The University reserves the right to offer a short-term contract appointment, or a probationary appointment rather than an appointment with immediate tenure.

Conditions of Appointment & application forms can be obtained from the Association of Commonwealth Universities (Apptd), Gordon Square, London WC1H 0PF.

Applications close on November 1978.

7898(A)

Clinical Trials Scientist

Astra Chemicals Limited is the U.K. subsidiary of Scandinavia's largest pharmaceutical group which specialises in the research, manufacture and marketing of cardiovascular agents, bronchodilators, psychotropics and local anaesthetics.

As part of our plans to extend our clinical trials operation in the U.K. we are now looking for a Clinical Trials Scientist to join our team at Astra's new offices in St Albans.

The objective of this function is to effectively design, monitor and report on clinical trials in support of Astra's pulmonary products. Full product training will be given both locally and in Sweden and the position involves travel mainly within the U.K. and occasionally overseas.

Candidates, aged 28 to 35, should be graduates in biological sciences or in pharmacy (preferably to doctorate level) with previous experience in the pharmaceutical industry.

An attractive salary will be negotiated plus choice of company car and other company benefits.

For further details telephone: (031) 225 2251. Letters of application together with C.V. should be addressed to:

Astra Clinical Research Unit, 65 Queen Street, Edinburgh EH2 4NA. 7934(A)

ASTRA

CLINICAL RESEARCH UNIT

MERSEY REGIONAL CENTRE FOR RADIOTHERAPY AND ONCOLOGY
Clatterbridge Hospital, Wirral, Merseyside, L63 4JY

RESEARCH FELLOW

(MEDICAL STATISTICIAN)

required to undertake the statistical work in a multi-disciplinary evaluation of previous, current and future methods of treatment for the malignant diseases of man. The Clinical Research Unit has been built recently to accommodate the Research Fellow and associate staff.

The person appointed will be responsible for organising the work of this new Unit, which will include analysis of the 260,000 records accumulated in the Regional Cancer Registry serviced by a computer-based system; the design and monitoring of clinical trials concerned with malignant disease, and the production of authoritative reports on the results of the management of malignant disease within the Region. He/she will be encouraged to conduct his/her own research and develop the existing computer-based data processing system.

Candidates should hold a first or higher degree in statistics, or hold a degree in medicine or science and have considerable statistical experience. Some knowledge of medical statistics and epidemiology is desirable. There will be facilities for postgraduate work for a higher degree. If suitably qualified, it is hoped that the successful candidate will develop the liaison with the Biostatistics Unit, Faculty of Medicine, University of Liverpool.

The salary will be in the range of £4,421 to £5,326 and the appointment is for 12 months in the first instance.

Further information can be obtained from: The Clinical Research Unit at the address above. Application forms must be returned within two weeks of the publication of this notice. 7946(A)

EEC PHARMACEUTICAL COMPANY

seeks

DIRECTOR OF RESEARCH

The successful candidate will report directly to the Managing Director and should have particular capabilities in:

- Creativity and initiative substantial experience in pharmaceutical research.
- Strong background in pharmacology.
- The company is medium sized, located in Paris and is already actively engaged in international development and licensing.
- Its research facilities employ approximately 100 people.
- Nationality indifferent, fluent English and French essential.

Please write to: SODECI, 140 Rue de la Croix Nivert, 75015 Paris (France). 7909(A)

Centro Internacional de Agricultura Tropical

CIAT Senior Staff Positions

The Centro Internacional de Agricultura Tropical is a nonprofit organisation devoted to agricultural development in the lowland tropics and is one of ten international centres supported by the Consultative Group for International Agricultural Research. The positions are:

Forages Explorer (Location: Cali, Colombia headquarters). This position is responsible for collecting grass and legume forage ecotypes from acid soil regions of the humid tropics as the main source of germplasm for CIAT's Beef Program; organising and conducting expeditions in cooperation with national institutions; processing, multiplying and maintaining a computerised inventory of forage germplasm. Qualifications: PhD or equivalent in forages or allied disciplines, competence in plant taxonomy, genetics, soils and climate; willingness to do extensive travel in remote areas and interest in collecting for developing improved tropical pastures; and ability to work with other scientists in an interdisciplinary, multi-national team.

Animal Scientist (Location: Carimagua Station, Eastern Plains of Colombia). This position is responsible for research at the herd level to develop and transfer improved beef production technologies based on improved forages in the savannahs of tropical America; for supervising regional studies on actual beef production systems, and for managing a 2500-head experimental herd. Qualifications: International-level animal scientist with PhD or equivalent with competence in both theoretical and practical knowledge of beef production systems in the tropics. Ability and willingness to work as a member of an interdisciplinary, multi-national team aimed at increasing beef production in acid soil regions of tropical America primarily through improved forage production. Must be willing to live in a rural experimental station.

Coordinator, Special Studies (Location: Cali, Colombia headquarters). This position requires an agricultural scientist with agronomic orientation. Responsibilities would include:

1. Supervision of research activities conducted by temporary staff (postdoctorals, visiting scientists) in the areas falling outside or across CIAT's commodity programmes, such as:
 - characterisation of land systems and climatological data for selected regions,
 - evaluation of improved cultivars of food crops and soil-conserving species which may play a role in improving productivity and resource conservation in small scale farming systems,
 - cross-commodity research in specialised disciplines (such as nematology, biochemical physiology, weed science, microbiology and others).

2. Conduct research in one or more of areas described above. Qualifications: PhD or equivalent in crop ecology, plant physiology, climatology, general agronomy or soil science and managerial experience and/or interest.

Coordinator, Data Services (Location: Cali, Colombia headquarters). Responsibilities include management of the Center's computer facilities and statistical services for research programmes, and supervision of six professionals plus clerical assistants. Computational equipment includes an in-house IBM 34 (64K) and telephonic access to an IBM 370/145 (VS1, 1024K). Activities will involve consultation on statistical design, development and interpretation, and development/adaptation of software in support of research activities. Communication at CIAT is in both Spanish and English. Qualifications: PhD or equivalent in biometrics or statistics with experience in agricultural science (or vice-versa) plus experience in operating and managing computers. He should be adept in the commonly used experimental designs, multi-variate analysis and mathematical programming including maximisation and simulation modelling.

Interested applicants should write to Dr K. O. Rachle, Associate Director General for Research; CIAT; Apartado Aereo 67-13; Cali, Colombia. 7967(A)

THE UNIVERSITY OF MANITOBA

invites applications and nominations for the position of

DEAN OF THE FACULTY OF AGRICULTURE

The Faculty of Agriculture at the University of Manitoba has a complement of 99 faculty and 150 support staff. It consists of six units, the Departments of Agricultural Engineering, Agricultural Economics, Animal Science, Food Science, Plant Science and Soil Science and the School of Agriculture responsible for approximately 774 undergraduate degree and diploma students and 206 graduate students in M.Sc. and Ph.D. programmes. The Faculty carries a full range of teaching and research responsibilities and extensive liaison activities with organisations and individuals in the agricultural community.

Candidates should have qualifications appropriate for appointment to a senior academic position and strong leadership ability. Demonstrated knowledge of and preferably experience in Canadian Agriculture will be an important consideration. Relevant administrative experience would be an asset.

The appointment is expected to commence September 1, 1979 and is for a renewable term of normally five to seven years.

Please address enquiries and recommendations to:

Provost R. A. Johnson, Chairman,
Selection Committee for Dean of Agriculture,
Room 202, Administrative Building,
University of Manitoba,
Winnipeg, Manitoba,
Canada R3T 2N2.

Applications will be received until December 18, 1978.

7916(A)

THE ANIMAL VIRUS RESEARCH INSTITUTE

PIRBRIGHT, WOKING, SURREY
Vaccine Research Department

Applications are invited for a senior appointment in the Vaccine Research Department from Biochemists to lead a section on problems relating to the development of methods for the characterisation of antigens such that the essential immunising component of different virus strains can be measured with precision, which could lead to reliable in-vitro estimates of potency. This topic is of fundamental importance and one of the growth points in Foot-and-Mouth Disease virus research. Good facilities are available, including provision for the production and purification of virus at the semi-industrial scale. Candidates should have a good Honours Degree plus several years' experience in this field and proven ability in the biochemical and analytical aspects of animal virus research.

Appointment will be in the P.S.O. grade (£6,609 to £8,461), housing available for married applicant. Further particulars and application forms from Secretary. 7936(A)

RESEARCH ASSOCIATES Ph.D. position INSTITUTE OF BIOLOGICAL SCIENCES, SYNTEX RESEARCH

available immediately to study the role of prostaglandins in kidney function, the relationship of prostaglandin structure to renal function and anti-hypertensive effects and to develop model systems to screen diuretic agents. Strong background in renal physiology essential; some knowledge of pharmacology of prostaglandins and anti-hypertensive drugs desirable. Appointment is for one or two years. Good benefits program. Syntex, a pioneer in the field of steroid chemistry and medicine, is an international pharmaceutical company whose continued growth requires a staff increase. For the highly motivated person this is an ideal opportunity for personal growth and advancement. Send résumé and names of three references to Dr John J. Bruno, Institute of Biological Sciences, Syntex Research, 3401 Hillview Avenue, Palo Alto, Ca 94304. Syntex is an equal opportunity employer m/f/h. 7937(A)

UNIVERSITY OF CAMBRIDGE SHELD PROFESSORSHIP OF PHARMACOLOGY

Applications invited for the above Chair and Headship of Department which is now vacant. Candidature limited to persons whose work is connected with any branch of Pharmacology except Clinical Pharmacology.

Proposed pensionable stipend £10,939 plus £578 for Headship of Department.

Applications (10 copies) marked 'Confidential' should be sent to the Secretary General of the Faculties, from whom further information may be obtained, at the General Board Office, The Old Schools, Cambridge CB2 1TT. Names of two referees may be submitted if desired. Closing date: November 15, 1978. 7945(A)

DERBYSHIRE AREA HEALTH AUTHORITY CENTRAL DERBYSHIRE DISTRICT

DERBY CITY HOSPITAL UTTOXETER ROAD, DERBY SCIENTIFIC OFFICER

Applications are invited for the post of Scientific Officer at Derby City Hospital. The appointee will be expected to partake in a research programme related to the immunological assessment of patients with malignant disease. Techniques involved would include lymphocyte culture, sub-typing and cell freezing.

Candidates should possess a degree or equivalent in a biological science.

Salary scale £2,991 to £4,275 per annum (entry point dependant on qualifications).

For further information about the post those interested should contact Mrs J. Clark, Scientific Officer, Oncology Laboratory (Derby) (0332) 40131, extension 329.

Application forms available from Sector Administrator, extension 428, to be returned by October 22, 1978.

Ref. No. 2156 7383(A)

Freshwater Biological Association RESEARCH ASSISTANT

Vacancy for Assistant Scientific Officer (ASO)—Scientific Officer (SO) to assist in work on the physiological ecology of planktonic algae and environmental factors of lakes, involving experimental and field studies. Two places at 'A' level (or equivalent) specialising in science, or a degree in biology, ecology, or environmental sciences.

Salary ASO £2,188 (age 18), £2,665 (age 21) to £3,303 (max.), SO £2,839 to £4,415 (max.).

Further details and application form from Secretary, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 0LP. Telephone Windermere (096 62) 2468. Closing date: November 10, 1978. 7968(A)

UNIVERSITY OF NEWCASTLE UPON TYNE LECTURER IN AGRICULTURAL AND ENVIRONMENTAL SCIENCE

Applications are invited for appointment to a newly established Lectureship in the Faculty of Agriculture from well qualified persons with research experience of either pollution control or landscape and biological conservation in the countryside. The main teaching duties will involve courses in the above subjects for the interdepartmental Honours Degree in Agricultural and Environmental Science.

Salary will be at an appropriate point on the Lecturers' scale: £3,883 to £7,754 per annum, according to age, qualifications and experience. Membership of the appropriate University superannuation scheme will be required.

Further particulars may be obtained from the Registrar, The University, Newcastle upon Tyne, NE1 7RU, with whom applications (3 copies), together with the names and addresses of three referees, should be lodged not later than December 1, 1978. Please quote reference N. 7957(A)

UNIVERSITY OF RHODESIA FACULTY OF SCIENCE Applications are invited for appointment as LECTURER OR SENIOR LECTURER

in
BOTANY. Applicants who
competent to teach in one
more of the following fields
be particularly welcome: Cr-
ganic botany, ecology or
physiology and anatomy
morphology

CHEMISTRY: Applicants
qualified to teach Inorganic
Analytical Chemistry will
be preferred.

Salary Scales (Approx. Sgs eqn
Senior Lecturer £7,549 by £29,
£8,428 by £311 to £9,983, Lec
Grade I £6,848 by £257 to £7
Lecturer Grade II £4,237 by £22
£5,337 by £257 to £6,622
Permanent Pensionable To
Family passages and allowance
wards transport of effects on app
ment Installation loan of up to
of one year's salary if required,
furnished University accommod
guaranteed for a period of at
three years for persons recruited
outside Rhodesia. Sabbatical I
and Triennial visits with travel al
ance. Superannuation and Mo
Aid Schemes

Short-term Contracts Pa
passages and allowance towards t
port of effects. Assistance with ac
modation, for persons recruited
outside Rhodesia

Applications (six copies) giving
details of personal particulars, qu
cations, experience and publica
together with the names and addr
of three referees should be sent to
Senior Assistant Registrar (S
University of Rhodesia, P.O.
MP 167, Mount Pleasant, Salub
Rhodesia. Overseas applicants sh
send an additional copy of
applications to the Association
Commonwealth Universities (Ap
36 Gordon Square, London W
OFF. Further particulars may be
tained from either of the above.

Closing date for receipt of ap
plications October 31, 1978

British subjects considering app
for posts in Rhodesia are urged
consult the Foreign and Com
wealth Office (telephone 233-4143
Off their nearest British Consular
7897C

UNIVERSITY OF OTAG Dunedin, New Zealand LECTURER OR SENIO LECTURER IN GEOPHYS

Applications are invited for
position of Lecturer or Senior Lec
in Geophysics in the Departmen
Geology.

Applicants should hold a F
degree or its equivalent and sh
preferably have had some
doctoral teaching or research
experience in solid-earth geophysics
appointee will be expected to dev
a geophysics programme of tea
and research within the Departmen
Geology. It is anticipated that
will contribute a short course
principles of geophysics to all ge
students at second-year level,
optional course on applied geoph
at third-year level, and an opt
advanced course or courses accor
to his/her own specialised inter
It is intended that the work of
appointee will be fully integrated
the existing programme of the De
ment.

SALARY SCALES:— Leo
NZ\$11,894 to \$14,488; Senior Lec
NZ\$14,983 to \$18,380 with a ba
\$17,145 per annum. (Salary scales
currently under review)

Further particulars are avail
from the Association of Comm
wealth Universities (Appts),
Gordon Square, London WC1H
or from the Registrar of the Un
ity. Applications close on Decem
13, 1978. 7972C

UNIVERSITY OF AUCKLAND New Zealand

Applications are invited for the following full-time teaching positions. Conditions of Appointment and Method of Application are available from the Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF. Applications, in accordance with 'Method of Application' will be accepted at any time up to the closing dates stated.

At present all salaries are supplemented by a General Wage Order of NZ\$365 per annum. For Lectureships, commencing salaries will be determined in accordance with qualifications and experience within the scale NZ\$11,529 per annum rising to NZ\$14,123 per annum by six annual increments. In normal circumstances a Lecturer whose services have proved satisfactory to the Council may expect in due course to be given the status of a Senior Lecturer.

GEOGRAPHY

Chair in Human Geography
(Re-advertisement)

Closing date extended to:
October 31, 1978

The position is one of two established Chairs in the Department of Geography. The vacancy arises because of the retirement of Professor K. B. Cumberland on May 31, 1978. The other Chair is held by Professor Paul Williams, who is at present Head of Department. Besides being experienced in University teaching, applicants should be active in research and have a substantial record of research publications. Candidates suitably qualified in any branch of Human Geography will be considered. At present professorial salaries are established within the range NZ\$22,647 to NZ\$28,823 per annum, having regard to the qualifications of the candidates concerned.

GEOGRAPHY

Lectureship

Closing date: November 14, 1978

Applicants should have qualifications within the field of Economic Geography. The Department is especially interested in candidates with expertise in behavioural analysis and/or regional development. Willingness to offer an area studies course would also be an advantage. An opportunity will be given to teach at both the undergraduate and graduate levels.

7920(A)

THE BRITISH COUNCIL

invites applications for the following post:

SENIOR LECTURER IN PLANT GENETICS (Sri Lanka)

University of Sri Lanka, Jaffna Campus
Candidates should hold a Ph.D., preferably in Plant Genetics and have at least 5 years teaching experience. Preferred age range: 30 plus.

Salary: £5,681 to £7,707 p.a. Benefits: Personal allowance, free furnished accommodation and other benefits. Two-year Formula contract.

Return fares are paid. Local contracts are guaranteed by the British Council.

Please, write briefly stating qualifications and length of appropriate experience, quoting reference number 78 PU 102 and title of post, for further details and application form to The British Council, 65 Davies Street, London W1Y 2AA. 7969(A)

Junior Laboratory Scientific Officer required in Department of Microbiology. Some experience of cell culture and virology preferable. Salary according to age and qualifications. Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 1RT, quoting Ref. M.C.4. 7890(A)

unilever research

Chemistry Graduates

Applications are invited from recent chemistry graduates for the following two vacancies in our Confectionery Fats Section.

Research Assistant (Ref: CH-480A)

Tasks:

To assist a Scientist in a number of projects in the confectionery fats field involving chemical techniques, blend formulation, computer modelling and general computations. The job also covers the field of product formulation and assessment.

Applicants for this post should preferably be experienced in the use of computers.

Research Assistant (Ref: 481A)

Tasks:

To determine the basic physical chemistry of confectionery fats using techniques such as X-ray Diffraction and Thermal Analysis. The complex interaction of different fats with one another will be studied and evaluated to determine their suitability for use in the confectionery industry.

Colworth Laboratory

Colworth Laboratory is a large, modern, very comprehensively equipped Food R & D Centre servicing Unilever Companies in the UK (Batchelors, Birds-Eye, Walls) and abroad. It is situated in a pleasant country estate ten miles north of Bedford. Salaries and fringe benefits are at a level expected of a large international company and includes assistance with moving expenses where appropriate.

Please write quoting reference number and giving details of career history to:

Miss J. Kenny,
Personnel Officer,
Unilever Research,
Colworth Laboratory,
Colworth House,
SHARNBROOK,
Bedford MK44 1LQ.



7894(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF VETERINARY ANATOMY RESEARCH ASSOCIATE (Re-advertisement)

Applications are invited for the post of Research Associate in the Department of Veterinary Anatomy to work on an A.R.C. funded project on prenatal muscle development in the pig.

The project is for three years starting on December 1, 1978, or shortly thereafter. Applicants should be graduates in Veterinary Medicine, Zoology, Agriculture or a related subject.

The successful applicant will have the opportunity to register for a higher degree.

Applications, before November 3, 1978, together with a curriculum vitae and the names of two referees, should be sent to Dr N. C. Stickland, Department of Veterinary Anatomy, Royal (Dick) School of Veterinary Studies, Edinburgh EH9 1QH, from whom further particulars may be obtained. Please quote Reference 5049. 7941(A)

STRATHCLYDE REGIONAL COUNCIL

DEPARTMENT OF EDUCATION FURTHER EDUCATION

Applications are invited for the undernoted post at Glasgow College of Technology, Cowcaddens Road, Glasgow G4 0BA.

LECTURERS 'A' in BIOCHEMISTRY AND PHYSIOLOGY

Honours Degree in relevant subject(s), preferably with postgraduate qualification, to contribute to the general teaching commitment of the Department including B.Sc. Applied Biology (with Honours) and B.A. Nursing Studies.

Salary scale: Lecturer 'A' £4,029 to £7,116 Bar £7,638. Placing on the salary scale will be given for relevant experience.

Forms of application and further particulars can be obtained from the College to whom completed application forms should be returned not later than October 27, 1978.

EDWARD MILLER, Director of Education.

7961(A)

Research Pharmacologist

Pharmacology Department

Roche Products Limited is part of a major international pharmaceutical company based in Switzerland and is itself one of the leaders in the industry in the United Kingdom.

Applications are invited for a post of Research Pharmacologist in our anti-thrombosis group. The Pharmacology Department is responsible for carrying out research and screening work aimed at the development of new drugs as part of the programme of inter-disciplinary groups.

The work involves routine experimentation and screening and would suit someone who has recently obtained a first degree in Pharmacology or who has a year or so of relevant industrial experience.

Our Pharmacology Department is located in modern laboratories in Welwyn Garden City where the working conditions and facilities are excellent. Conditions of Service include a Christmas Award, 4 weeks' annual holiday, pension fund membership, free BUPA membership and subsidised restaurant. In appropriate cases assistance will be given towards relocation.

If you would like to apply for this post, please write briefly or telephone for an application form (Welwyn Garden 28128) to Mrs Clewley, Research Personnel Department, quoting reference RD33, Roche Products Limited, P.O. Box 8, Welwyn Garden City, Herts. AL7 3AY.

7935(A)


ROCHE

UNIVERSITY OF EDINBURGH

Applications are invited for the post of

RESEARCH FELLOW

in a study of human breast tissue, supported by a three year grant from the Medical Research Council. The project involves electron microscopy, immunohistochemistry and cell kinetics. Applicants should have a Ph.D. or equivalent. Salary Scale is Range 1A, and the appointment will commence as soon as possible. Closing date November 2, 1978.

Applications, including names and addresses of three referees should be sent to Dr T. J. Anderson, Department of Pathology, University of Edinburgh Medical School, Teviot Place, Edinburgh EH8 9AG. Please quote Reference 5057 7915(A)

ROYAL FREE HOSPITAL MEDICAL LABORATORY SCIENTIFIC OFFICER

required to undertake Biochemical investigations for home dialysis patients being treated by our Renal Unit. Applicants should be experienced in Biochemistry and Immunology.

Salary on scale £3,615 per annum to £5,034 per annum inc. Application forms from Personnel Department, Royal Free Hospital, Pond Street, Hampstead NW3. Tel. 794 0500, ext. 4286.

Closing date October 20, 1978 7899(A)

ST. BARTHOLOMEW'S HOSPITAL

London EC1A 7BE

SCIENTIFIC OFFICER

is required to join the D.H.S.S. Toxicology Unit. This Unit carries out investigations into the possible hazards to health by chemicals in food, cosmetics and other consumer products in the environment.

Applicants should hold a degree in one of the biological sciences and preferably have some relevant experience.

Salary according to experience £3,345 to £5,253 inclusive.

Application forms and job description from Personnel Department, telephone 01-600 9000, ext. 3186, quoting Ref. PTA/182.

7950(A)

oxford polytechnic

Lecturer—

Senior Lecturer in Physical Anthropology

(Salary £4,101—(bar)—£7,572)

Applications are invited from those with suitable qualifications to teach Human Ethology, Primatology and Primate Ethology at Honours degree level. Teaching experience and publications in Anthropology and Primate Ethology are preferable.

Lecturer—

Senior Lecturer in Experimental Psychology

(Salary £4,101—(bar)—£7,572)

Candidates for this appointment should be competent to teach Experimental Psychology to Honours degree level, specialising in the areas of Learning and Physiological Psychology. Candidates should have teaching interests in other areas and be ready to teach on introductory courses and to supervise final year projects. Experience in on-line control of experiments and data processing is desirable.

Applications, including a curriculum vitae, and the names and addresses of three referees, should be sent to the Head of Department of Social Studies, Oxford Polytechnic, Oxford. OX3 0BP, from whom further details and application forms may be obtained.

7955(A)

POSTDOCTORAL FELLOW UNIVERSITY OF ALBERTA

Applications are invited from recent Ph.D.'s for the position of Postdoctoral Fellow effective April 1, 1979. Applicants should have a strong background in biochemistry, biophysics or a related area and an interest in conducting research on the organisation and dynamics of lipids in biological membranes and/or on the lipid modulation of the activity of membranous enzymes. Salary is \$11,600 for the first year and \$12,720 for the second year. Send a curriculum vitae and three letters of reference to Dr R. N. McElhaney, Department of Biochemistry, University of Alberta, Edmonton, Alberta, Canada T6G 2H7 7944(A)

UNIVERSITY COLLEGE LONDON

DEPARTMENT OF GENETICS AND BIOMETRY RESEARCH ASSISTANT

Applications are invited for this post for work on human gene mapping. It involves linkage studies on enzyme polymorphisms, blood groups and chromosome markers, and a general knowledge of biochemistry and interest in genetical statistics would be desirable.

The post is available for three years, financed by the M.R.C., and the salary will be £3,664 with annual increments. Candidates should have a good Honours Degree and it will be possible to register for a higher degree. Applications, together with the names of two referees, should be sent as soon as possible to Professor E. B. Robson, Department of Genetics and Biometry, The Galton Laboratory, Wolfson House, 4, Stephenson Way, London NW1 2HE. 7894(A)

UNIVERSITY OF LIVERPOOL

Applications are invited for

THREE POSTS OF POSTDOCTORAL SENIOR RESEARCH ASSISTANT

tenable for a period up to three years:

(a) To join a group working on plant sterol biosynthesis. The applicant will study the mechanisms of U sterol side chain methylation reactions using plant tissue culture or cell-free preparations.

Appointments (2) and (3) are to join a group working on the steroidal insect moulting hormone (ecdysteroids) in insects and nematodes.

(b) An ORGANIC CHEMIST/BIOCHEMIST is required to investigate the biosynthesis and metabolism of the ecdysteroids in insects. Experience of steroid or terpene chemistry will be an advantage.

(c) A BIOCHEMIST/PHYSIOLOGIST is required to investigate the structure and metabolism of ecdysteroids in nematodes.

The appointments are tenable for a date to be arranged at an initial salary within the range £3,883-£4,382 per annum plus U Benefits.

Applications, together with names of two referees, should be received as soon as possible.

The Registrar,
The University,
P.O. Box 147,
Liverpool,
L69 3BX

from whom further particulars may be obtained. Quote Ref: RV/957 A, or C/N 7953(A)

SCIENTIFIC SERVICES - TIDAL WATERS

The Tidal Waters Section of the Directorate of Scientific Services of the Welsh Water Authority is involved in the assessment of coastal pollution resulting from the discharge of domestic and industrial waste around the Welsh Coastline and in the evaluation of remedial measures where necessary.

Applications are invited for two posts within this section:

SCIENTIFIC OFFICER

(MARINE OUTFALLS)

Grade 6 (£5133-£5493) or Grade 7 (£5682-£6004) including supplement
(REFERENCE 192)

Within the Tidal Waters section is a survey team responsible for carrying out detailed hydrographic, chemical, and biological surveys to assist in the formulation of these remedial measures. The team requires an Officer to be responsible for the organisation and implementation of the scientific aspects of the Authority's hydrographic surveys, for the evaluation of data so obtained, and for the production of scientific reports clearly outlining the results of the surveys and the options available for sewage disposal.

The successful applicant will have a higher degree or equivalent in physical oceanography (or other relevant discipline) with considerable experience of coastal hydrographic survey techniques and computer orientated evaluation of survey data. Experience of mathematical modelling of coastal circulation and dispersion would be advantageous.

Both posts are based at the Authority's Headquarters in Brecon. The Scientific Officer's post will involve a considerable amount of travelling in Wales and a Regular User Car Allowance will be paid in this post.

Both appointments are temporary for six months in the first instance, but will be subject to consideration for permanency on the forthcoming re-organisation of the Authority.

Further particulars of the post together with application forms, may be obtained from:



WELSH WATER AUTHORITY
AWDURDOD DWR CYMRU

ASSISTANT PERSONNEL OFFICER
WELSH WATER AUTHORITY
CAMBRIAN WAY
BRECON POWYS
LD3 7HP.

Tel: Brecon (0874) 3181.

7929(A)

Closing date for applications: 16th November 1978

IMPERIAL COLLEGE TECHNICIAN (GRADE 3)

Required in a research group working in the field of genetic engineering. The successful applicant would be expected to purify restriction endonucleases and assist with a variety of DNA sequencing problems. Experience of a good theoretical knowledge of techniques of protein purification is desirable. Salary in the range of £3,153 to £3,525 inclusive (under review). Superannuation scheme. Appointment for one year initially but extension likely.

Applications to Departmental Superintendent, Department of Biochemistry, Imperial College, London W7 or telephone 01-889 3111, Ext. 119 for an application form.

7919(A)

EAST BIRMINGHAM HEALTH DISTRICT EAST BIRMINGHAM HOSPITAL BIOCHEMIST, BASIC GRADE (HAEMATOLOGY)

Supervise routine biochemical investigations including abnormal hemoglobins, red cell enzymes, and oxygen dissociation curves. In addition assistance with the current research projects involving erythropoietin and ferritin will be encouraged. Salary range £2,991 to £4,275 depending on qualifications.

For further information apply to Dr C. H. B. Gyde, Department of Haematology, East Birmingham Hospital, Birmingham B9 5ST. Tel. 21-772 4311 Ext. 4282.

Applications, stating details of age, experience and naming two referees. Mrs G. Potts, Assistant Hospital Administrator, East Birmingham Hospital, Bordesley Green East, Birmingham B9 5ST.

7928(A)

UNIVERSITY OF GLASGOW DEPARTMENT OF BIOCHEMISTRY POSTDOCTORAL RESEARCH ASSISTANT

A Postdoctoral Research Assistant is required for a period of up to 14 months for an S.R.C. supported project on structure-function relationships in transfer RNA. Research experience in Biochemistry or a related subject (and preferably in enzymology or nucleic acid structure) is required. Salary will be within Range 1A of the salary scales for Research and Analogous Staff, £3,883 to £4,382 per annum, according to qualifications, age and experience. Appropriate Superannuation Scheme will apply.

Applications enclosing a curriculum vitae and the names of two referees should be submitted by October 31, 1978, to Dr J. P. Goddard, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ.

In reply please quote Ref. No 4251M.

7942(A)

UNIVERSITY OF EDINBURGH DEPARTMENT OF BACTERIOLOGY Postdoctoral Fellow/ Research Associate

required to work on a Cancer Research Campaign sponsored project in tumour immunology. Experience in working with tumours or in the field of cellular immunology would be desirable. Salary on National Range 1A.

Schd. c.v. to Dr W. H. McBride, Department of Bacteriology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland. Please quote Reference 5058.

7940(A)

HERIOT-WATT UNIVERSITY DEPARTMENT OF BREWING AND BIOLOGICAL SCIENCES RESEARCH ASSOCIATE

Applications are invited for the post of Research Associate tenable for up to three years for research on yeast cell walls, and yeast cell growth and division under the direction of Professor D. J. Manners and Dr J. H. Duffus.

The salary will be on the Range 1A scale £3,384 to £6,555 per annum (under review).

Informal inquiries may be made to Professor D. J. Manners.

Application forms and further particulars are available from the Staff Officer, Heriot-Watt University, Chambers Street, Edinburgh EH1 1HX, to whom completed forms should be returned by Saturday, November 11, 1978.

7911(A)

UNIVERSITY OF OXFORD DEPARTMENT OF HUMAN ANATOMY TECHNICIAN GRADE III Salary £2,688 to £3,060 p.a.

A Technician is required to assist with histology and immunocytochemistry, preparation of solutions and animals, and maintenance of a laboratory. Work is in connection with brain research. Knowledge of the cryostat and stereotaxic techniques would be an advantage. The appointment is for a fixed term ending July 31, 1981.

Applications should be made in writing without delay together with the names and addresses of two referees to The Administrator, Department of Human Anatomy, South Parks Road, Oxford, OX1 3QX.

7914(A)

UNIVERSITY COLLEGE OF NORTH WALES BANGOR DEPARTMENT OF TECHNICIAN GRADE 4

£2,955 to £3,402 per annum

Applications are invited for the post of Histology Technician in the Department of Zoology.

The main duties will be the preparation and sectioning of material for examination in the light microscope together with some assistance in the laboratory.

Qualifications required are O.N.C. or an equivalent qualification.

Applications (two copies), giving details of age, education and experience, together with the names and addresses of two referees should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd LL57 2DG, by not later than October 16, 1978.

7926(A)

UNIVERSITY OF EDINBURGH LECTURESHIP IN PHYSIOLOGY

Applicants must have a good Honours degree in Physiology and at least 3 years research experience. Salary £3,883 to £7,754 per annum. The post is available from January 1, 1979.

Further particulars may be obtained from the Secretary to the University, Old College, South Bridge, Edinburgh EH8 9YL, with whom applications (6 copies) including a full curriculum vitae and the names and addresses of three referees should be lodged not later than October 31, 1978. Please quote Reference 1074.

7939(A)

Metabolism Chemist

The Plant Protection Division of ICI wishes to recruit someone with post graduate research experience in chemistry, to lead a team studying the metabolism of pesticides.

Applicants must have experience of the use of separative techniques such as glc and hplc, together with a sound mass spectrometry background. Ideally, we need someone who has worked successfully on problems involved with the isolation and identification of small quantities of sample and who has an understanding of the use of labelled isotopes.

The successful candidate will be innovative and will have the ability to motivate and direct a research team. It is also necessary to work constructively in a multi-disciplinary project team.

We offer competitive salaries and there is a Company Profit Sharing Scheme. Assistance with removal expenses and house purchase may also be available. The Research Station is situated on a pleasant farm estate between Bracknell and Maidenhead in Berkshire.

If you are interested in this vacancy please write or telephone for an application form to

Mr D Spence
Personnel Officer
ICI Plant Protection Division
Jealott's Hill Research Station
BRACKNELL RG12 6EY
Berkshire

Telephone Bracknell 24701 Ext:3350



Plant Protection
Division

7923(A)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF OCEANOGRAPHY Applications are invited for the post of LECTURER

in the Department of Oceanography from January 1, 1979, or as soon as possible thereafter. The person appointed will be expected to teach and carry out research in Marine Chemistry.

The initial salary will be within the range £3,883 to £5,367 per annum on a scale rising to £7,754 per annum.

Applications, together with the names of three referees, should be received not later than November 14, 1978, by The Registrar, The University, P.O. Box 147, Liverpool, L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/958/N 7951(A)

BIOCHEMIST—MOLECULAR BIOLOGIST

The Department of Biological Sciences, Carnegie-Mellon University invites applications from molecular biologists whose research interests lie in the area of nucleic acid structure and/or function. Candidates should have postdoctoral experience. The position is at the assistant professor level, although higher appointments will be considered. Duties include teaching at the undergraduate and graduate level as well as maintaining an active research programme. Applicants should forward their curriculum vitae, three or more letters of reference and a brief résumé of research plans to Search Committee (2), Department of Biological Sciences, Carnegie-Mellon University, 4400 Fifth Ave., Pittsburgh, PA 15213. Carnegie-Mellon University is an Equal Opportunity/Affirmative Action Employer 7349(A)

THE UNIVERSITY OF MANCHESTER RESEARCH ASSISTANT IN BIOCHEMICAL MORPHOLOGY

Applications are invited for this post, tenable up to two years, from candidates with a good degree in chemistry or biochemistry, and a strong interest in research, to join a team working on the structure and function of connective tissue. Salary range per annum £3,384 to £4,382. Applications should be sent as soon as possible to Professor J. E. Scott, Department of Medical Biochemistry, Stopford Building, Manchester M13 9PT 7893(A)

TECHNICIAN/RESEARCH OFFICER

required by our Human Tumour Immunology Group at University College Hospital Medical School, London, W.C.1 to assist in the studying of host defence mechanisms against infection in patients being treated with cytotoxic chemotherapy for malignant disease.

H.N.C. or degree with experience in immunology and tissue culture. Salary range £3,615 to £5,256.

For application form telephone or write to Miss S. M. Hurley, Imperial Cancer Research Fund, Lincoln's Inn Fields, London WC2 on 242 0200, ext 305, quoting reference 303/78 7895(A)

Scientific Publishers, NW London, require P/A for admin/editorial work, part or full time. Please reply to Box No 7906, 3 Dyers Buildings, Holborn EC1N 2NR 7900(A)

THE ZOOLOGICAL SOCIETY OF LONDON

seeks a

CURATOR OF MAMMALS

To manage the Society's Collection of Mammals at Regent's Park, to advise the Society in regard to mammals exhibited both there and at Whipsnade Park and to collaborate in research carried out in the Society's Institute of Zoology.

Applicants should be professional zoologists or veterinary surgeons with a wide knowledge of mammals, and should be competent to carry out research in the field of biology in which they have specialised. They should have had management and administrative experience.

Salary is related to the Senior Lecturer/Reader scale of London University and will be in the scale £6,792 to £9,264 plus London Allowance of £435, with entry point according to qualifications and experience. Pension arrangements are under the Universities Superannuation Scheme and the Universities Supplementary Dependents Pension Scheme. Residential accommodation may be available.

Applications should be made in writing, with details of qualifications and experience and the names of two referees, to:

The Secretary
The Zoological Society of London
Regent's Park
London NW1 4RY

as soon as possible, and not later than November 30, 1978.

7923(A)

ANIMAL DISEASES RESEARCH ASSOCIATION H.S.O./S.O.

A vacancy will shortly occur in the Institute's Physiology Department for a person to be responsible for the running of a Foetal Studies Unit and participation in the experimental programme involving surgical, radioactive isotope procedures and chemical analyses. Previous experience desirable but not essential. Applicants should have an ordinary degree, H.N.C. or equivalent with experience or Hons degree in an appropriate subject. Five years relevant post qualifying experience is required for an appointment at H.S.O. level. Salary Scales—S.O. £2,839 to £4,415, H.S.O. £4,101 to £5,448 with entry according to the qualifications and experience of the successful applicant. Non-contributory superannuation scheme.

Applications giving the names of two referees should be sent to The Secretary, Animal Diseases Research Association, Moredun Institute, 408 Gilmerton Road, Edinburgh EH7 7JH from whom further particulars may also be obtained 7386(A)

UNIVERSITY OF NEWCASTLE UPON TYNE THE DENTAL SCHOOL LECTURER IN ORAL ANATOMY

Applications are invited from dental or science graduates for the whole-time post of Lecturer in Oral Anatomy tenable from January 1, 1979. The successful candidate will be required to take part in the teaching and research programme of the Department of Oral Anatomy which is located in the recently opened new Dental School providing excellent modern teaching and research facilities. Commencing salary will be according to age, qualifications and experience on one of the following scales for dental graduates either Pre-Clinical General Grade £5,129 to £7,754 per annum or Pre-Clinical Higher Grade £7,268 to £8,257 per annum; for science graduates Lecturers' Non-Medical Grade £3,883 to £7,754 per annum. Membership of the appropriate University superannuation scheme will be required.

Further particulars may be obtained from the Registrar, The University, Newcastle upon Tyne, NE1 7RU, with whom applications (three copies), together with the names and addresses of three persons to whom reference may be made, should be lodged not later than November 6, 1978. Please quote reference W 7960(A)

THE UNIVERSITY of Manchester, Technician Grade 3. Applicants should be competent in general histological techniques. The post will also provide experience in electron microscopy, histochemistry and autoradiography. Applicants should have an O.N.C. or equivalent qualification and a minimum of three years' relevant laboratory experience.

Salary scale £2,688 to £3,060 p.a. (scale under review)

Applications in writing, giving details of age, education and qualifications, together with technical experience and the names of two referees, should be made to Professor P. F. Harris, Director of the Anatomical Laboratories, Stopford Building, Oxford Road, Manchester M13 9PT. Closing date October 27, 1978 7932(A)

LIVERPOOL POLYTECHNIC DEPARTMENT OF CHEMISTRY/BIOCHEMISTRY A RESEARCH ASSISTANT/TECHNICIAN

is required for a one-year project funded by the North West Cancer Research Fund entitled: "Studies on the possible carcinogenicity of novel steroids obtained from bile acids by bacterial transformation".

The work involves testing possible carcinogenic compounds using the Ames test, and a test for transformation of mammalian cells in culture.

Applicants should have a degree or technical experience in a Biological Science including experience of Microbiology.

Salary £2,511 plus £312 p.a. For further information apply to Dr R. Bilton or Dr E. A. Haslem, tel 051-207 3581 ext 26.

Application forms and further particulars from the Personnel Office, Liverpool Polytechnic, Richmond House, 1 Rumford Place, Liverpool L3 9RH. Tel 051-227 5581 ext. 43. Please quote reference LP/231 7971(A)

CENTRO DE INVESTIGACIONES Y DE ESTUDIOS AVANZADOS Mexico City, Mexico

Two positions exist in the Department of Genetics and Molecular Biology for Assistant/Associate Professors working with pro- or eukaryotic microorganisms. Laboratory facilities are excellent, teaching responsibilities minimal, knowledge of Spanish is not necessary. Applicants should possess a Ph.D. Send curriculum vitae, names of three referees, synopsis of research and reprints to Dr Jose Rui Herrera, Depto de Genética y Biología Molecular, C.I.E.A. del I.P.N. Apartado Postal 14-740, Mexico 14 D.F. 7281(A)

CONFERENCES

GORDON RESEARCH CONFERENCES
"Frontiers of Science"

1979 WINTER SCHEDULE

Miramar Hotel, Santa Barbara, California

January 8-12

Electrochemistry

CO₂ Fixation by
Green Plants**January 15-19**

Polymers

Crystal Growth

January 22-26

Biology of Aging

Deformation and Failure
Mechanisms in Polymers
and Composites**January 29-February 2**

Hormone Action

Agricultural Science

February 5-9Chemistry and Physics
of Isotopes**Conference Fees:**

Conferee (registration, room, meals)	..	\$200.00
Guest (room, meals)		\$150.00

Program:

The complete program will be published in the October 20, 1978, issue of *Science*.

Requests for applications or for additional information should be addressed to:

Dr. Alexander M. Crulckshank, Director
Gordon Research Conference
Pastore Chemical Laboratory
University of Rhode Island
Kingston, RI 02881

7905(C)

ENDOCRINOLOGY '79

The 7th biennial International Conference organised by the Endocrine Unit at the Royal Postgraduate Medical School will be held at the Royal College of Physicians in London from July 16-19, 1979.

The programme will centre on recent work in recent advances in molecular biology (especially with recombinant DNA)—implications for endocrinology, endocrinology of calcium metabolism and metabolic bone disease (to include calcitonin, vitamin D and osteoporosis), brain peptides including enkephalin and LHRH, prostaglandins and thromboxanes; cellular biology of hormone secretion; and peptide chemistry.

The speakers invited include Boyer, Goodman, Rich, Guillemin, Kosterlitz, Iversen, Vane, Martin, Orci, Whedon, Nordin, Haussler and Norman.

Further information from The Conference Secretary—Mrs Phyllida Lindsay Endocrinology '79 (NI), Royal Postgraduate Medical School, Du Cane Road, London W12 0HS, U.K.

7933(C)

PERSONAL

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- Session II. Cholinergic System
- Session III. Biogenic Amines
- Session IV. Other Endogenous Neuroactive Compounds
- Session V. Neuroleptics
- Session VI. Histochemical and Morphological Aspects
- Round Table Discussion 7903(C)

FELLOWSHIP

French Government Scholarships for further study and postgraduate research in France in Pure and Applied Science, Technology, Medicine, Social Sciences, Economics, Geography and Agriculture are offered for the academic year 1979-1980. Candidates, who must be United Kingdom citizens, should be university graduates or have equivalent qualifications. Closing date December 20, 1978. Details and application forms from Scientific Department, French Embassy, 41 Parkside, Knightsbridge, LONDON SW1X 7JP. Citizens of the Commonwealth and of other countries should apply direct to French Embassies in their respective countries 7904(H).

AWARDS

Awards for RESEARCH INTO TROPICAL RESPIRATORY DISEASES

The Wellcome Trust wishes to encourage research into diseases of the respiratory system, including asthma, occurring in the tropics. A competitive award of up to £100,000 is offered for a period of not more than five years. Preference will be given to proposals which demonstrate an interdisciplinary approach and especially those which include sustained collaboration with research centres in the tropics.

Applications for smaller awards will also be considered and grants may also be made to the runners up. *These awards are additional to the Trustees' normal arrangements for supporting tropical medical research.*

Those wishing to apply should send a two-page summary of their objectives and proposals for research, including a brief estimate of the anticipated costs, for preliminary consideration. The principal scientist should also send a curriculum vitae.

Applications should be addressed to Mr. M. A. F. Barren, The Wellcome Trust, 1 Park Square West, London NW1 4LJ, and reach the Trust by 8th December 1978.

The Wellcome Trust

7966(N)

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ASSISTANTSHIP

UNIVERSITY OF HULL
DEPARTMENT OF APPLIED PHYSICS

RESEARCH ASSISTANTSHIP
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Applications are invited for a research assistantship tenable for a period of up to two years in the first instance with the possibility of renewal. The research assistant will be involved in the study of magnetic particle inspection testing and ultrasonic testing of a wide variety of industrial components, in collaboration with the Health and Safety Executive, Nuclear Inspectorate.

Salary will be on the scale £3,384 to £4,882 (under review) together with U.S.S. benefits.

Applications, which should include the names of two referees, should be sent by November 1, 1978 to: Dr S. B. Palmer, Department of Applied Physics, University of Hull, Hull HU6 7RX, from whom further particulars may be obtained 7927(P).

The University of British Columbia

Department of Chemistry

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Graduate Teaching Assistantships are available for candidates with high academic records who wish to pursue further studies towards the M.Sc. and Ph.D. Degrees in Chemistry. Excellent research facilities are available in all areas of modern Chemical Science, ranging from Bio-Inorganic Chemistry to Chemical Physics. For first year students the minimum stipend, including the summer research assistantship, is anticipated to be \$7,000. Students entering the programme with a Master's degree will receive a higher stipend. In addition, many special merit awards are made to those graduate teaching assistants who show excellence in the performance of their duties. Numerous graduate scholarships are also available for students with outstanding academic records.

Application forms and further particulars may be obtained from the undersigned:

Professor C. A. McDowell
Department of Chemistry
The University of British Columbia
2075 Westbrook Mall
Vancouver, B.C.
Canada V6T 1W5

7938(P)

FELLOWSHIPS

UNIVERSITY COLLEGE CORK POSTDOCTORAL RESEARCH FELLOWSHIP (VIROLOGY/CELL BIOLOGY)

A vacancy exists for a

Virologist/Cell Biologist

to study tumour virus (SV40) host cell interaction through the use of temperature sensitive host cell mutants. The project is funded by the Irish Cancer Society and the appointment will be initially for one year with a possible extension to three years.

Commencing salary will be in the region of £3,600 per annum.
Starting date: by arrangement.

Applications with curriculum vitae and the names and addresses of two referees should be sent **as soon as possible** to Dr J. K. Collins, Virology Unit, Department of Microbiology/Dairy & Food Microbiology, University College, Cork, Ireland.

7954(E)

POSTDOCTORAL FELLOWSHIP

(three years)

available to recent Ph.D. interested in developing **RAPID-RESPONSE CARCINOGENESIS ASSAYS**. Needs good degree in Biology or Biochemistry, ability to work independently. Send application with brief research proposal, curriculum vitae and three references to: Dr Murray Goodall, Cancer Research Unit, University of Otago, Box 913, DUNEDIN, NEW ZEALAND.

7900(E)

NEWNHAM COLLEGE CAMBRIDGE RESEARCH FELLOWSHIPS

The College, acting as part of a Group, invites applications from women educated in Great Britain or the Commonwealth for one or more Research Fellowships which will normally be tenable from October 1, 1979.

Further details, together with application forms (which must be completed and returned not later than November 1, 1978) may be obtained from the Secretary, C.I.A.O., Kellet Lodge, Tennis Court Road, Cambridge CB2 1QJ.

7930(F)

THE QUEEN'S UNIVERSITY of Belfast

RESEARCH FELLOWSHIP

DEPARTMENT OF BIOCHEMISTRY

This post, available until September, 1980, involves research on biochemical aspects of measles and canine distemper viruses. The work is supported by a grant from the Medical Research Council and is concerned with how defective interfering particles may be involved in establishing persistent infections in tissue cultured cells. Candidates should have some years experience in the field of nucleic acids (particularly RNA) and proteins. Experience in handling bacterial or animal viruses would be an advantage.

Salary scale (under review) is £3,384 to £5,130 with superannuation, initial placing depending on age and experience.

Applications giving names and addresses of two referees should be sent to the Personnel Officer, The Queen's University of Belfast BT7 1NN, Northern Ireland. Closing date: October 27, 1978.

7952(E)

UNIVERSITY OF KEELE RESEARCH FELLOWSHIPS IN BIOCHEMISTRY

Two fellowships are available with Professor J. B. Lloyd in the Biochemistry Research Laboratory, Department of Biological Sciences.

1. For work on the nutrition of the early mammalian embryo (M.R.C. supported). Experience with tissue culture techniques and radioisotopes an advantage.

2. For work on the pinocytic uptake and lysosomal digestion of potentially biodegradable synthetic polymers (Cancer Research Campaign). Experience of cell fractionation and enzyme assays an advantage.

Both posts could be filled at either postdoctoral (salary scale 1A, from £3,883 p.a.) or predoctoral (salary scale 1B, from £3,384 p.a.) level.

Further details from Professor J. B. Lloyd, The University, Keele, Staffs., ST5 5BG (tel: 0782 621111, ext. 65), to whom applications, accompanied by a curriculum vitae and the names of two referees, should be sent as soon as possible.

7956(E)

UNIVERSITY OF SOUTHAMPTON HUMAN MORPHOLOGY FELLOWSHIP

Applications are invited from medical or science graduates for a fellowship which will involve a maximum of six hours teaching a week. Previous teaching/research experience, although not essential, would be an advantage. The successful applicant will take part in teaching/demonstrating anatomy and/or histology to medical undergraduates, join a research group studying some immunological aspects of pregnancy, and will be encouraged to register (if appropriate) for a higher degree.

Salary within range £3,883 to £6,555. Minimum at age 26, £4,382 per annum. U.S.S. benefits.

For further details contact Dr S. Peel, Human Morphology, Medical and Biological Sciences Building, Bassett Crescent East, Southampton. Applications giving date of birth, curriculum vitae and the names of three referees should be sent to Mrs. P. Vaughan-Smith, The University, Southampton SO9 5NH by October 27, 1978. Please quote reference 1030/R/Na.

7892(E)

VISITING FELLOWSHIPS FOR RESEARCH IN SOLID EARTH AND ATMOSPHERIC SCIENCE

One year awards offered by C.I.R.E.S., a Research Institute sponsored by N.O.A.A. and the University of Colorado. Stipend is scaled to experience, and the program is open to scientists of all countries. Applications for the 1979-80 academic year awards should be sent to The Director C.I.R.E.S. Visiting Fellows Program, University of Colorado, Boulder Colorado 80309 U.S.A., before February 1, and should include names of three referees, a vitae and publications list, and a brief outline of the proposed research.

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7387(E)

GIRTON COLLEGE CAMBRIDGE

Applications are invited for a scientific research fellowship open to graduates of any university and tenable for three years from October 1, 1979. The applications should be in the field of Mathematics, Natural Sciences, Geography and allied subjects.

The fellowship is to the value of £2,475 per annum to a candidate with a Ph.D. and £2,255 per annum to a candidate who has not completed a Ph.D. The fellowship is pensionable under U.S.S.

Particulars are available from the Secretary to the Council, Girton College, Cambridge CB3 0JG to whom applications should be sent by November 15, 1978.

7913(E)

STUDENTSHIPS

UNIVERSITY OF WARWICK C.A.S.E. STUDENTSHIP—

KINETICS OF DIAZONIUM SALT REACTIONS BY FAST REACTION METHODS

Applications are invited for a three year S.R.C. (C.A.S.E.) Research Studentship in the Department of Chemistry and Molecular Sciences, starting October 1, 1978, and supervised by Dr T. G. Kemp in collaboration with Dr J. Atherton (I.C.I. Organics). The programme will involve a kinetic study of the decomposition and coupling reactions of ArN_2^+ ions using stopped-flow spectrophotometry combined with a rapid-scanning spectrometer. Applicants should hold or expect to hold at least an Upper Second Class honours degree in chemistry. Further details may be obtained from Dr Kemp, Department of Chemistry and Molecular Sciences. Applications, with the names of two referees, as soon as possible, to the Academic Registrar, University of Warwick, Coventry CV4 7AL. Please quote Ref. No: PG/40/78.

7891(F)

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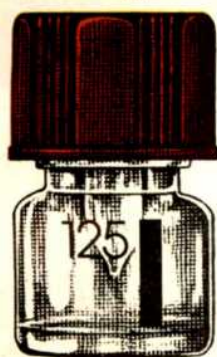
Further information and application forms may be obtained from the Registrar, University of Sydney, NSW 2006, Australia, with whom applications close on November 17, 1978.

7973(H)

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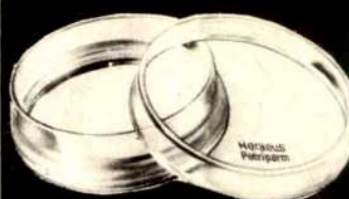
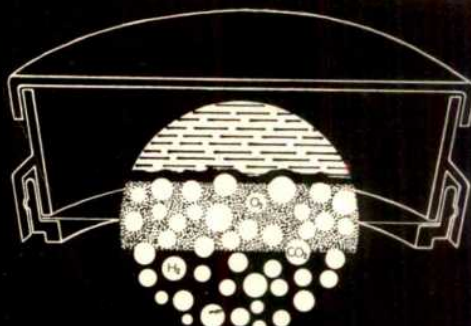
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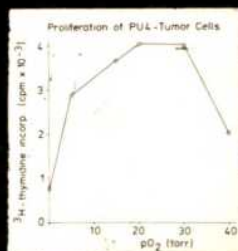
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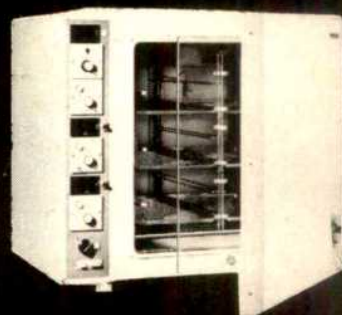
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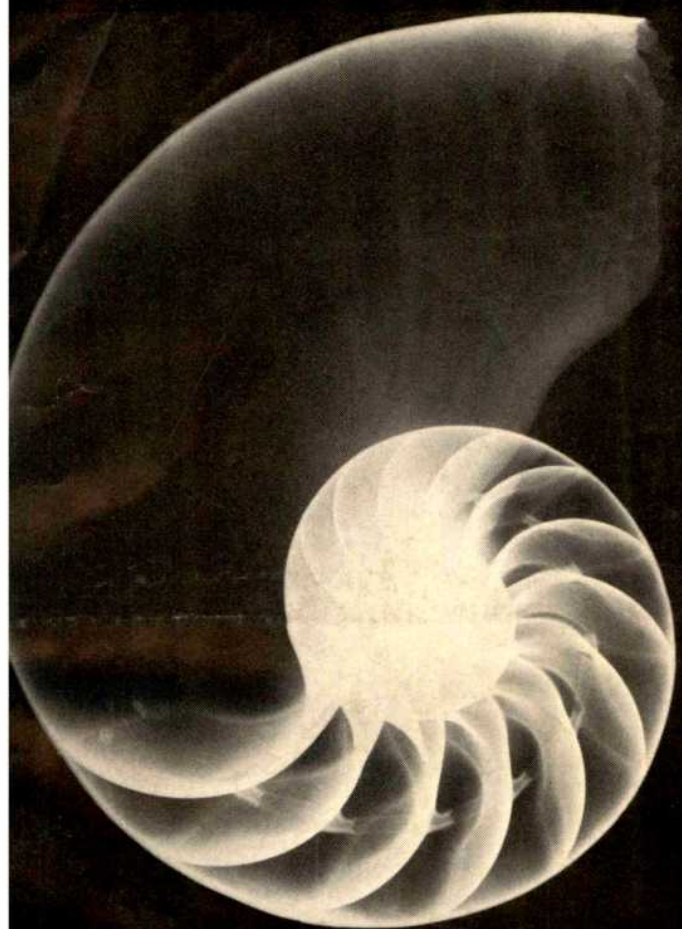
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Study of growth lines of fossil Nautilus shells shows that the number of days per lunar month has increased dramatically during the past 420 Myr.
(Natural History Photographic Agency)

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nature

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What's new

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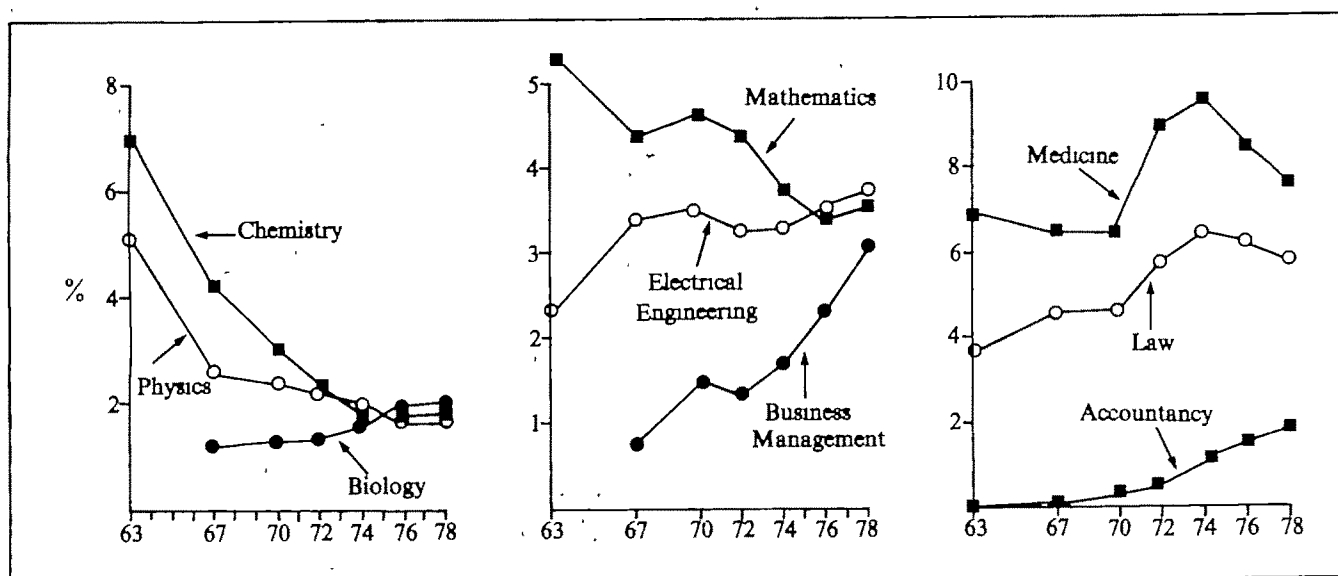
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nature

19 October 1978

Popular science



THE above three graphs show changing patterns in popularity of various subjects amongst applicants to British universities over a period of fifteen years. The figures have been compiled from annual reports of the Universities Central Council on Admissions (UCCA), culminating in the Statistical Supplement to the Fifteenth Report, published recently. These reports have over the years consistently listed the number of candidates applying through the UCCA scheme for entry to any British university (excluding the Open University, University College, Buckingham and to a lesser extent three Scottish universities) by their first choice for study.

There were some changes in subject headings between 1963 and 1967 which mean that comparisons must be made a little cautiously (although there seems no evidence that the subject headings are seriously incompatible). Since 1967 the headings have remained the same, however, so there is no reason to doubt that year-by-year comparisons can be made. The figures are percentages of all applicants naming particular subjects as first choice. The total number of applicants in 1963 was 51,596 and in 1978 is about 180,000. Between 1963 and 1967 the number rose to 90,952; it has risen more slowly since then. Only one subject, chemistry, actually has less applicants in 1978 than in 1963.

As with all statistics, some cautions have to be given. These are not figures for admissions to university, so they do not reflect actual changes in size of intake. Very many students naming a preferred subject are happy, or at least willing, to be accepted for another subject. Furthermore, the figures say nothing about the quality of candidates. Many of those who apply simply do not have the necessary qualifications, and cannot be regarded in the final analysis as serious candidates for a university. Also amongst those who are serious candidates, there is probably substantial variation from subject to subject in the quality of applicants. Finally, the figures for 1978 are only estimates, though accurate ones.

What can be claimed is that the graphs represent fairly well the ambitions of 17-year-olds over a period in which a much greater number of students have been able to contemplate university education, and in which society's attitudes to various professions have changed substantially. Perhaps there are two points worth underlining. The first is that the vast expansion in educational opportunities in the 1960s did not stimulate in any way a proportional increase in the number of those doing the 'central' sciences. Secondly, contrary to popular belief, enthusiasm for engineering courses has actually grown fairly steadily for many years. □

Power-failure knocks out Seasat

A POWER-FAILURE thought to have been caused by a short circuit has cut off all communication with the experimental ocean-monitoring satellite Seasat-A, launched less than four months ago by the National Aeronautics and Space Administration.

Attempts by NASA's Jet Propulsion Laboratory in California to re-establish contact with the satellite have so far been unsuccessful. And it is feared that the satellite, which cost \$75 million to put into orbit, and was expected to continue to provide data for up to three years, may now have to be written off.

Seasat is primarily a "proof of concept" satellite designed to test the performance of a set of microwave sensors, including in particular a synthetic aperture radar which is able to gather data on the behaviour of the ocean's surface even through dense cloud.

In the four months since the satellite was launched considerable quantities of data have been successfully gathered and stored for future processing. NASA officials claim that the results processed so far indicate that the behaviour of the equipment has been "amazingly good".

However the apparently short life of the satellite has been a major disappointment to scientists who had expected to make use of a full range of data in a series of oceanographic and meteorological studies not only in the US, but also in Canada and Europe.

Canada's Department of Energy and Mines, for example, which is collecting Seasat data at a receiving station in Newfoundland, was in the process of a major survey of arctic

ice flows using simultaneous data from aircraft and the satellite. The survey will now have to be completed using aircraft data alone; and although the impact on the project is not likely to be substantial, one scientist described the loss of further Seasat data as a "considerable blow".

The trouble occurred last Monday when Seasat was in its 1503rd orbit. According to NASA officials the satellite seemed to be behaving normally when it passed over the receiving station at Orrora in Australia; however when it came within sight of the next station at Santiago in Chile, it was discovered that the batteries were discharging at full rate.

As the voltage dropped rapidly, various sensors began to shut down, and within a few minutes the satellite ceased to respond to commands sent up from ground control stations. A little later communication from the satellite to Earth also cut out.

Laser beams bounced off special reflectors on the bottom of the satellite as it passed over Bermuda showed that it was still on course. However continued attempts to get the satellite to switch off all non-essential equipment, in the hope that solar cells might recharge the batteries, have apparently so far met with little success.

Various bursts of data thought to be from Seasat were reported last week by several receiving stations, including one at Oakhanger near London, but it turned out that these had been transmitted from the earth-monitoring satellite Landsat-3.

The cause of the power failure is not yet known (although NASA officials were denying rumours that it might



have been knocked out by a Soviet anti-satellite weapon). However late last week it was discovered that a recorder left on for practice purposes at Oakhanger had been receiving signals at the time of the failure, and its data is now being studied to see if any clues to the short-circuit could be found.

Because of tight budget restrictions, Seasat-A is a one-off satellite with no back-up available. Although officially planned to remain in orbit for only one year, it had been supplied with sufficient resources to last for three. The soonest that a follow-up satellite could be launched, even with Congressional approval, would be 1983. □

Carter outlines future strategy for space science

PRESIDENT Carter has officially confirmed that there are unlikely to be any further US "space spectacles" such as the Apollo project during the next decade, and that while the administration remains committed to a strong space science programme, this must remain sufficiently flexible to absorb financial constraints "when conditions warrant".

However, included among future space science goals will, in addition to planetary studies currently underway, be more detailed exploration of Saturn and its moons and rings, and the reconnaissance of comets and asteroids.

These decisions were announced last week following a policy review of civilian space policy carried out by an

interagency committee set up four months ago under the chairmanship of Dr Frank Press, director of the Office of Science and Technology Policy.

The president's statement contains few surprises, and reflects the more cautious—some would say conservative—path that the National Aeronautics and Space Administration has been pursuing in the past few years. Its general message is that if there is to be a major expansion in space activities, the main stimulus is to come from private industry rather than the federal government.

As for space science, the statement lays down broad principles rather than specific objectives. Thus President Carter says that US space policy will

reflect a balanced strategy of application, science and technology development which will "assure American scientific and technological leadership in space for the security and welfare of the nation".

Such a policy will, he says, "emphasise space science and exploration in a manner that retains the challenge and excitement and permits the nation to retain the vitality of its space technology base, yet provides short-term flexibility to impose fiscal constraints when conditions warrant."

The president's statement says that it is neither feasible nor necessary at this time to commit the United States to a "high-challenge space engineering initiative" comparable to Apollo, and

that the now declining costs of the Space Shuttle development programme gives the administration "the flexibility to give greater attention to new space applications and exploration, continue programs at present levels, or contract them." Funds to cover these will be "adequate."

Turning to space science, the president says that in addition to continuing a vigorous programme of planetary explorations to understand the origin and evolution of the solar system, the US will utilise the space telescope and free-flying satellites to usher in "a new era of astronomy" which will include the exploration of interstellar molecules, quasars, pulsars and black holes.

The most controversial part of the statement comes in the section covering space applications, and in particular the future direction of earth monitoring systems such as LANDSAT. The

administration has been under Congressional pressure for some time to come up with a clear set of policy goals and long-term objectives.

Last month, for example, Senator Adlai Stevenson, chairman of the Senate subcommittee on science and space, introduced a bill which would require the president to present Congress annually with a five year forward-plan clearly laying out the administration's space objectives and how it proposed to meet them.

However, so far the administration has backed away from any firm commitment of this nature. And last week's statement merely says that, in the case of remote sensing systems, NASA will chair an interagency task force "to examine options" for an integrated system, and that a comprehensive plan covering all aspects of remote sensing "will be explored." □

World climate conference thrown open to social scientists

WHEN the World Climate Conference meets in Geneva in February next, it will involve some 400 participants instead of the 100 or so originally envisaged by its organisers at the World Meteorological Organisation (WMO). So now the social as well as the physical sciences can be represented—a move in line with the new trend of thinking on the role of science in the United Nations system. This broader participation also underlines the main objective of the conference "to examine the impact of climate on various aspects of human welfare, and especially of climatic fluctuations on all time scales".

WMO's Secretary General Dr D. A. Davies points out that the effects of cold spells and droughts have in recent years presented grave problems affecting energy supplies and agricultural output even in the most highly developed countries. At the other end of the economic ladder, the effects of the disaster caused originally by the drought in the Sahel in the early 1970s

are still causing grave concern. So this effort to bring about a "meeting of the minds" between the meteorologists and the behavioural scientists, biologists, economists and others in relevant disciplines is certainly not being made a moment too soon.

Nevertheless no great scientific surprises are expected to emerge from next February's meeting. As WMO sees it, hard evidence does little to support many of the disaster hypotheses of the doom-watchers. It is admitted, however, that there is one major exception: the problem of increasing CO₂ in the atmosphere. This is something in which WMO is seriously engaged, and is considered important enough for a major global effort to be made in the immediate future: the proposal is to use a wide network of regional observing stations, together with a smaller number of permanent baseline stations sited so as to cover the whole of the Earth's surface.

Peter Collins

"Erosion of spirit" in British science

THE morale of academic research scientists in British universities is at a low ebb, according to the Trustees of the Nuffield Foundation, a British charitable organisation which supports university research.

Writing in the annual report of the foundation, the trustees claim to observe "what appears to be an erosion of the spirit in scientific and medical research at British universities and polytechnics". The trustees base this

assertion on the fact that the Nuffield Foundation "has received many fewer applications of high quality for research grants in science and medicine than would have been expected if university departments were bursting with ideas and needing only financial resources to carry them through". The morale of the younger members of the community is particularly affected, says the report.

The trustees select lack of growth in

ICSU investigates refused visas

ICSU the International Council of Scientific Unions—is to investigate the complaint of a group of Israeli geneticists who failed to receive visas for the fourteenth International Congress of Genetics, held in Moscow last August.

Originally, 33 members of the Israeli Society of Genetics registered for the congress and received a registration number, indicating that their application was accepted. However, a month before the congress, visas had not yet arrived.

On 10 August, the Israeli Society of Genetics cabled Professor Ralph Riley, secretary of the International Genetics Federation, suggesting that the federation withdraw its sponsorship of the congress. This, it appeared, was impossible, both because the federation was financially committed, and also because it was feared such an action would "throw Russian genetics into turmoil again".

Eventually 18 visas arrived—restricted to the exact dates and place of the congress and with no provision for accompanying spouses nor for participation in the traditional post-congress excursions. At this the Israeli Society of Genetics decided to recommend its members not to attend—a recommendation which was duly followed. Instead, two delegates attended to put the Israeli case formally.

After various unofficial discussions, the case was raised at the representative council of the federation, and a resolution was passed, mild in tone, but demanding that "steps should be taken to ensure that all applicants to further congresses receive their visas in due time".

Last month, when the ICSU general assembly met in Athens, the resolution was brought to the attention of the Committee for the Free Circulation of Scientists. The Committee, which includes among its membership a representative of the Soviet Academy of Sciences, has undertaken to look into the matter.

Vera Rich

the universities as the main culprit: "younger people . . . can no longer set their sights on what until quite recently was the chief objective of young creative scientists—to be so successful in their chosen field as to make a mark not only in the scientific literature but on the pattern of research in their institutions."

The foundation offers to help—if anyone can come up with a practical proposal. □

Brazil's scientists fan doubts over energy priorities

David Dickson describes how problems with Brazil's nuclear programme have highlighted tensions between the government and the scientific community

ONE of the major issues that Brazil's new president will have to face when he takes power after next month's elections is whether the country should make a major shift in its energy strategy.

Officially the central component of this strategy remains a firm commitment to the rapid development of nuclear power, a programme under which the country is scheduled to possess nine power stations by 1990, and to be meeting 40% of its energy needs with nuclear power by the year 2000.

Criticism of the programme by Brazil's scientific community on technical, economic and political grounds appears so far to have been virtually ignored by the country's military leaders. And external criticism, such as the US's attempts to dissuade Germany from selling Brazil a re-processing plant on the basis that it would increase the danger of the proliferation of nuclear weapons, has hardened the nation's resolve to pursue its nuclear goal.

However, three factors are now putting strong pressure on Brazil's leadership to revise its current stance. The first involves problems and delays that have already occurred in the nuclear programme itself; in particular the extra construction work that has been made necessary by the discovery of geologic instabilities at present reactor sites.

The second is the rapidly increasing costs of the programme. Partly a result of the costs of the extra design and construction work, and partly due to the declining value of the cruzeiro against the Deutschmark, nuclear

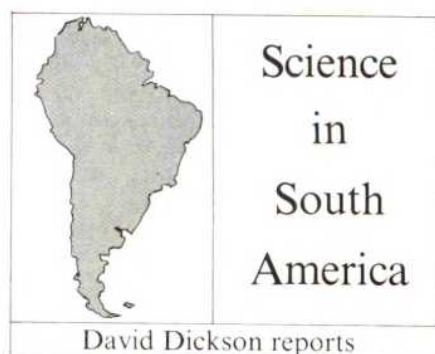
power is becoming a much less attractive proposition on economic grounds when compared to more conventional sources such as hydropower.

The third factor is the success that has already been achieved in Brazil's crash programme to substitute the use of ethyl alcohol for petroleum. Unlike nuclear power, the alcohol programme is well on the way to meeting its initial target of 4 billion litres a year by 1980; a further source of pride is that it has been based almost entirely on indigenous research and development. In many of Brazil's major cities cars now use a mixture of 20% alcohol, and by 1981 one-sixth of all new cars are planned to run on alcohol alone.

At the very least, some of Brazil's scientists now argue, the \$1.6 billion alcohol programme has removed the worst pressures of the energy crisis which had had such a dampening effect on Brazil's previously explosive economic growth, thus giving the country more time to consider the details of its nuclear future.

"Most scientists and industrialists would probably prefer a more modest programme in which they could play a dominant role and acquire the necessary technology" according to Professor Jose Goldemberg, director of Sao Paulo University's Institute of Physics, who has suggested that a prototype reactor working on the thorium cycle might be the most appropriate way forward.

Others go further to suggest that, given the vast potential of the nation's renewable energy sources—in particular in the fields of hydropower, solar



energy and the use of biomass—the most desirable option from both a technical and a social point of view would be for Brazil to drop its nuclear programme altogether.

The oil crisis of 1973 hit the country particularly hard. Unlike neighbouring Venezuela, it has little petroleum reserves, and intensive prospecting has been relatively unfruitful; 83% of its petroleum is now imported, at an annual cost of \$4 billion.

Even before the crisis, Brazil had committed itself to nuclear energy, signing an agreement with the US firm Westinghouse to build a 625 megawatt reactor on the coast at Angra dos Reis, 115 miles south-west of Rio de Janeiro, in 1969.

Construction work started in 1972, but has been plagued with problems. These range from an embarrassing fire last year, which caused \$10 million of damage to equipment and raised awkward questions about construction procedures and security, to the discovery that the site was on top of a rock platform that was slowly being pushed into the sea—and that a retaining wall was therefore needed to shield it from the pressure of earth moving down a nearby mountainside.

Other problems have been experienced with the subsequent contract for the construction of eight 1,300 megawatt reactors which Brazil signed with



Cars in Sao Paulo now run on a mixture of 20% alcohol



It's all right, sir. It's only suffering from a hangover!



Plagued with problems: Brazil's first nuclear power station under construction

West Germany in 1975. The package included not only equipment, materials and technical assistance, but also covered the construction of an enrichment plant and a reprocessing plant, providing, it had been hoped, the basis for Brazil's nuclear power programme to become self-sufficient.

It has been the inclusion of the enrichment and the reprocessing plants that have so far attracted the most outside attention. In addition to the Carter administration's attempts to block the sale of the latter, the Dutch government almost refused to allow the supply of enriched fuel from the European Urenco consortium because of concern over safety arrangements.

Such arguments, however, have few supporters inside Brazil. The military rulers have accused the US of interfering in another country's sovereignty, the same reason given for refusing to sign the Non-Proliferation Treaty; and even the government's political opponents, while expressing concern on the safety issue, have rallied behind it in rejecting outside pressure as "technological imperialism."

However, criticism of other aspects of the programme has been far from absent. Three issues in particular have attracted the attention of Brazil's scientific community, among whom the physicists have been especially vocal.

The first criticism is that, in buying the technology as a total package with no alteration permitted to the German design—even though the construction of parts will slowly be taken over by Brazilian contractors—no real transfer of technology will have taken place, and the Brazilians will have learned little about reactor design.

"The technological aspect of nuclear energy is vitally important, and we would like to learn how to develop our own technology so that we can develop a truly independent programme," says Professor Jose Moreira of the Institute of Physics, who claims that the short period spent in Germany by most of those sent to learn about nuclear tech-

nology means that little transfer of knowledge is likely to take place.

The second criticism relates to the proposed method for enriching uranium. The Germans have proposed using the as-yet unproven Becker jet-nozzle process. Research results, however, have so far been disappointing, and have led to the crack that Brazil is paying with money it does not have for technology that Germany does not possess.

Both of these relate to the third, broader criticism; that in developing its nuclear programme, the Brazilian government has taken little account of what its own scientists could or might be able to offer, and has thus neglected the opportunity to build a nuclear power industry truly independent of foreign interests.

Professor Moreira points out, for example, that at present Brazil has about 800 PhDs in nuclear physics, more than enough to establish a viable reactor research community, but that universities and technical institutes have been virtually ignored in the government's nuclear plans.

Similarly a group of physicists recently produced a report on "Panorama and Perspectives on physics in Brazil", which criticised the lack of interaction between the government nuclear company Nuclebras and the physics community, claiming that this appeared to be a deliberate policy, and pointing out that the considerable scientific resources of Nuclebras were not available to outside scientists.

The situation has historical roots. Back in the late 1960s when the nuclear plans were being drawn up, universities were a focus of opposition to the military government's authoritarian policies, and many staff members—including scientists such as Dr Leite Lopes, then scientific director of the Brazilian Centre for Research in Physics, and now professor of particle physics theory at the University of Strasbourg—lost their jobs.

A reluctance at the time to provide university research funds for a subject

as sensitive as nuclear energy is therefore little surprising. And the government even disbanded the main research group in the country, the Institute of Radioactive Research at the Federal University of Minas Gerais.

One consequence of the lack of involvement of universities, however, has been the reported difficulty of attracting scientists of sufficient calibre into the nuclear programme. For example it is claimed—although difficult to confirm, since the whole programme is shrouded in secrecy—that this is one reason why government laboratories have had such difficulty in developing the enrichment technique, even on an experimental basis.

"More than the petrol crisis, what is obstructing Brazilian development is the lack of human resources," Professor Jose Ellis Ripe, a physicist from the University of Campinas, held a conference last month on physics education. This, he said, was the result both of a low investment in education, including in particular education in the basic sciences, and a preference for investing government money in areas that promised a quick return.

One area of slightly less sophisticated technology in which Brazilian scientists and technologists have been able to get a firm grip has been in electric power transmission. Brazil and Paraguay are currently building what will be the largest hydroelectric dam in the world on the Parana river, eventually producing 12.6 gigawatts; Brazil has agreed to buy any power that Paraguay does not need, but since the two countries' electricity supplies are at 50 and 60 cycles a second respectively, Brazil is transmitting the power to its urban centres as direct current before reconverting it to alternating current.

Factors such as the need for smaller cables means that high voltage direct current transmission becomes more economical over a distance of about 700 kilometers. And engineers are now putting together ambitious plans to use such techniques to transmit hydro-power 2000 kilometres from the Amazon basin to the country's industrialised areas in the south, whose energy needs had previously stimulated the nuclear power programme.

But the area which has given the greatest boost to Brazil's self-confidence in its research capabilities has been the alcohol programme. From modest beginnings only a few years ago, the research programme has mushroomed under the personal support of President Geisel to a position in which several hundred separate projects are planned or operating, with a total budget of \$65 million over the next two years.

Even US scientists admit that this is an area of energy research and deve-

lopment in which Brazil has established an advantage over all other countries, and there has been much international interest—for example from scientists in West Germany and Scandinavia—in the programme and its results.

Reception of "gasohol" by consumers has been mixed; there are complaints of timing problems—the streets of São Paulo are filled with frequent back-fires—of difficulties in starting on cold mornings (overcome by the addition of a small pre-heater) and of an increase in fuel consumption.

Research scientists at the Aeronautical Technical Center near São Paulo, where much of the enthusiasm initially came from and where there are currently 34 separate programmes looking at engine design, insist that such problems are relatively minor, and that the technical feasibility of using alcohol in petrol on a large scale has now been clearly established.

In other places, much research is being carried out into the supply side. Initially the prime candidate for transformation into alcohol had been manioc, a root vegetable widely used by Brazil's native Indian which grows in poor soil with relatively little water—two characteristics of Brazil's Northeast region in which social conditions are worst, and needs are greatest.

However at present the main emphasis is being placed on methods of producing alcohol from sugar cane. Although unlike manioc, which can be harvested at any time, cane can only be collected for six months of the year, it is considerably easier to extract the sugar required for fermentation, and the outside sheath, known as bagaco, can also be burnt to provide the energy for the fermentation process.

Government officials, their appetite whetted by the apparent success of the alcohol programme, are now busy following up the potential uses of other biological materials. One prime candidate under consideration is the nut of babacu palm tree, at present used almost exclusively for its seeds, which are extracted from the nut's core and crushed to produce their oil.

Plans are now being studied to use each of the various parts of the nut for different purposes; the seeds to make palm nut oil, the core to make charcoal and gas, the shell to make alcohol, and the skin for primary combustion.

In addition to building the self-confidence of the research community, the whole field of alternative energy sources is proving to be a fruitful outlet for university scientists who had been previously frustrated in attempts to link their work to socially useful objectives.

The involvement which started with criticism of the government's nuclear

policy is now being channelled by some into more pragmatic goals. This process has been encouraged by the availability of funds from the federal government, which has recently set up an office in the Ministry of Mines and Energy, under the title of Project Ipiranga, to centralise research efforts on non-conventional sources of energy.

A group of physicists at the Federal University of Rio de Janeiro, for example, under the direction of Dr Luiz Pinguelli, is now looking at the energy options facing state power companies.

Similarly Professor Goldemberg and Professor Moreira in São Paulo are looking at ways of increasing the efficiency of the conversion processes applied to biological materials. In particular, they are investigating both theoretically and experimentally ways of using fluidised bed combustion to increase the efficiency of burning the sugar-cane bagaco which they claim could be used as an important source of electricity for other domestic and industrial purposes.

But the more general sense of frustration and exclusion remains. Many scientists, for example, are now calling on the government to permit the return of those of their colleagues who had previously been expelled, claiming that the country needs to do what it can do build a base that can shore up its ailing economy.

There have also been requests for greater participation in decision making on technological issues, as well as in the assessment of the general direction of bodies such as the National Research Council, Nuclebras, and even the Ministry of Education.

So far, despite the slight loosening of earlier restrictions, there has been little response to such requests. And in energy policy at least, there remains the feeling that political interests continue to play a dominant role.

One reason for the switch from

manioc to sugar cane in the alcohol programme, for example, was that despite the social advantages of the former in bringing work and income to the country's most depressed areas, the dramatic decline in world sugar prices in 1973/74 meant that the large sugar-cane producers needed new outlets for their produce, and pushed for their inclusion in the programme.

Nor are established interests far below the surface in the nuclear energy field. Thus there has recently been a scandal involving the close connections between the Minister of Trade and Industry and the owner of a previously obscure engineering company which was awarded, without any competitive bidding, the contract for the construction work for the first two German reactors, Angra 2 and 3.

Furthermore Brazil can expect heavy pressure from Germany if its attempts to back out—as Iran is now reported to be contemplating—from its previous commitment, even though, according to Mr Arnaldo Barbalho, president of the state-owned electricity company Eletrobras, "the agreement speaks only of the purchase of up to eight nuclear power stations."

Finally with Argentina, Brazil's main economic competitor in the South American continent, already successfully operating one reactor and building more, any suggestion of cutting back on nuclear plans is unlikely to be warmly received in military circles.

Last month the Brazilian congress, largely under pressure from the opposition party MDB, agreed to set up in the Senate a parliamentary commission of inquiry into the country's nuclear policy. Some scientists feel this could be an opportunity to explore the viability of a major shift towards alternative, mainly renewable energy sources. The technical potential has been demonstrated; the political will is now required. □

Argentina plans nuclear reprocessing plant

ARGENTINA is soon to start construction work on an experimental plutonium reprocessing plant, and could be operating a complete nuclear fuel cycle with domestic technology by 1990, according to Admiral Raul Castro Madero, President of the Atomic Energy Commission.

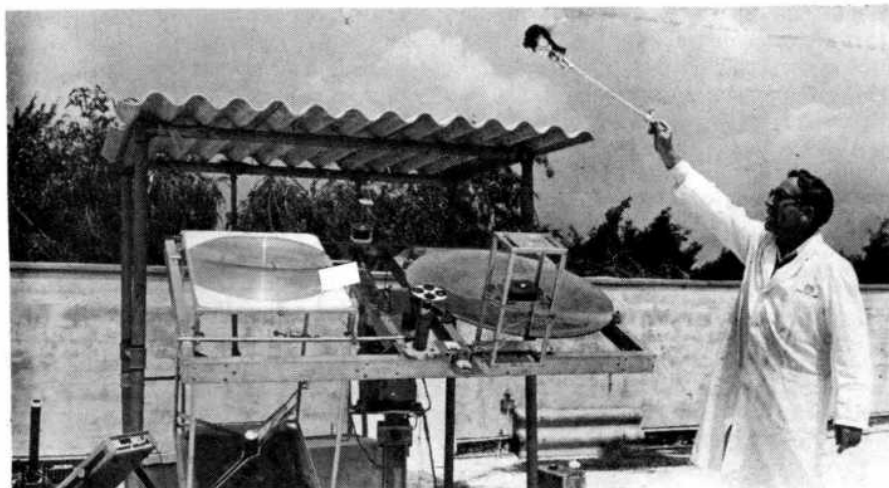
The reprocessing plant will be situated in the grounds of the Ezeiza Atomic Centre outside Buenos Aires. A laboratory scale reprocessing plant was built at Ezeiza in the early 1960s, and is said to have produced a few milligrams of plutonium before it was dismantled.

According to a report in the

Washington Post, Argentina is also planning to start production of a heavy water plant. The country already operates a natural uranium reactor—the only nuclear reactor presently operating in South America—and this would give it the capacity to produce nuclear fuel using domestic technology.

Since Argentina has not signed the nuclear non-proliferation treaty, it would be able to produce weapons-grade plutonium free of international safeguards. However, Admiral Madero said in an interview that the country had "no need for nuclear weapons". □

Israel's place in the sun



Israel's abundance of sun and scientists has led to great interest in the application of solar energy, writes **Vera Rich**

ISRAEL, it may be claimed, has had an interest in solar power dating back some 3,500 years—when Joshua ordered the Sun to stand still! But today there is a boom in the more scientific uses of solar energy in the country.

The modern era of Israeli solar power dates back to 1911, when a mining engineer from Siberia, Moshe Novomeiskii, first visited the Dead Sea. Novomeiskii realised that the combination of arid atmosphere and virtually unlimited sunlight (the annual rainfall is about 5 cm) would make the extraction of mineral salts by evaporation commercial. By 1930, the first plant, at the northern end of the Dead Sea, was in operation, and in 1934, a second plant was opened at S'dom, at the southern end of the sea, where the terrain is suitable for the construction of very large evaporation pans.

Following partition in 1948, the northern plant came into Jordanian hands, and was later destroyed; but the S'dom plant has become the basis of Israel's potash and chemical industry. The evaporation pan system now covers 130 km² and has a capacity sufficient to produce 1,250,000 to 1,400,000 tons of phosphate a year, much of which is exported, as well as bromine compounds and magnesium and sodium chlorides. The solar energy consumed in the evaporation process is estimated to be equivalent to more than Israel's annual output of electricity.

For some years, the Dead Sea works remained Israel's only effort in the use of solar energy. Then, about 20 years ago, the first solar-powered hot-water systems began to go into operation. At

the present time, even the prefabricated homes of the small Negev settlements have roof-top water heaters (two solar panels and a drum), and it is estimated that some 35% of the population enjoys solar hot-water—a total which would doubtless be higher had not certain local authorities banned roof-top storage tanks for aesthetic reasons.

Following the oil crisis of the early 1970s, and in view of the fact that Israel has so far located only minor deposits of oil and gas within the "green line" (the pre-1967 frontier), there is now a boom in all types of solar energy exploitation. At virtually every institute of higher education in Israel, a number of solar energy projects are going forward, in any available corner. Even the spacious Weizmann Institute seems to find it impossible to house all its solar team in an appropriate building, and one can find solar energy work going on, for example, in the basement of the animal breeding centre!

Broadly speaking, solar energy research falls into four main sections:

- passive domestic systems: water heaters, space heating and air conditioning;
- production of electricity from solar energy;
- use of photosynthesis and related processes to "fix" solar energy, and
- miscellaneous applications.

Research into passive domestic systems is now well advanced, and ranges from the development (at the S'de Boquer Desert Research Centre) of lightweight collector panels which the householder can install himself, to detailed investigations of the technical and economic problems of central

heating systems for apartment houses and other large establishments. Such a survey was carried out at the Ben Gurion University, Beer-Sheva, using the students' hostels as a pilot system.

A number of heating/air conditioning systems are being studied. At S'de Boquer, a whole line of one-room 'houses' has been erected, to permit the variation and comparison of the structural parameters involved. The underlying concept of the S'de Boquer research is that the development of the Negev should guarantee a reasonable quality of life for those who settle there; comfortable and appropriate housing is therefore all-important.

The storage element in these systems is simply a rough-fill of local stones and pebbles, which considerably reduces the cost. A somewhat similar principle has been tried at the Government Agricultural Research Station at Vulcani, for the heating of chicken-houses, but with less success; largely because chicken-house heating requires a series of peaks, rather than a steady background heat, and it is difficult to work out an economic regime of operation. Similarly, solar drying of tobacco failed to give the right leaf colour. The Vulcani team are not, however, too disappointed; the research has taught them a good deal, they consider, in the management of solar energy, and the solar drying technique might yet prove commercially viable for herbs such as oregano and mint.

Active solar energy systems, producing electricity from solar power, present a more complex problem. Economically speaking, the main problem is the cost of the silicon cells. In the USA, investments of the order of several hundred million dollars have been made in attempts to reduce the cost of the cells, and have succeeded, over the last two years, in reducing the effective cost of solar-cell electricity from \$40 to some \$12/Watt.

At the Weizmann Institute, however, a different approach is used—high-concentration arrays, using plastic Fresnel lenses to concentrate the sunlight onto small, relatively low-cost cells, and a mechanical tracking system to keep the array directed towards the Sun.

Over the last five years, considerable efforts have been made to incorporate the latest technological developments into the fabrication of high-efficiency solar cells, so that, at present, arrays of the Weizmann type can compete with small diesel generators. In the opinion of Dr Yosef Mandelkorn, by 1985, the cost of high-concentration silicon arrays will be low enough to warrant their use in self-contained urban communities away from the main centres of population.

One very interesting development

Figure (above): a large on-line Sun-tracking system for solar energy experiments at the Weizmann Institute of Science, Rehovot. The system can drive 2 m² of solar concentrators

of this kind of array, he added, is the possibility of using solar cells for the simultaneous supply of electrical and thermal (hot-water) energy, by mounting the cells on a structure of pipes through which a heat-exchange liquid flows.

In Dr Mandelkorn's opinion, use of high-concentration arrays could reduce the cost of silicon cell power generation to \$2 to \$4/Watt in the very near future—a figure which most experts would, he said, consider "realistic".

A far more radical approach to solar power generation is that of Dr Branover of the Ben Gurion University, Beer-Sheva. His project, which is supported by the Israeli Ministry of Energy, uses solar power to heat a low-boiling point liquid to about 70 to 80 °C, so that its vapour mixes with a suitable liquid metal (mercury, gallium, Wurtz metal) and accelerates it to a velocity of some 30 to 40 m/sec.

At this velocity, the liquid enters the field of a permanent magnet (about 0.5 Tesla). Depending on the circuit parameters, with this velocity and field it is possible to generate a field of about 1V with a current strength of up to 1,000 A.

Low temperature magnetohydrodynamic (MHD) generation of this kind, says Dr Branover, offers many advantages; it has no moving parts save the liquid metal, and is hermetically closed once for all. By connecting several generators in series, it is hoped to produce direct current of around 1,000 A at 10 V.

Such a power source, says Dr Branover, could be used in the chemical industry for producing copper, magnesium and aluminium. It could also be used for charging batteries for electric cars, which could change their batteries at garages in the same way that they now fill up with petrol. This would facilitate the change over from petrol-driven to electric automobiles without disrupting either the infrastructure of garages nor the fiscal system (since governments could simply tax the batteries instead of the petrol); however, this, says Dr Branover, would not be practical before about 1990, since the onus is on the automobile

manufacturers to produce a suitable vehicle.

His low-temperature MHD plant should, however, be in operation, at least at the pilot stage, by 1982; all the preliminary calculations are done, and work is now going ahead on building the model.

Another imaginative use of solar energy as a power source is based on *Dunaliella*—the only life-form capable of surviving in the Dead Sea. This alga manages to survive the high osmotic pressure by producing glycerol, which can amount to 85% of its dry weight. Possibilities of the commercial production of *Dunaliella* are being worked out both by the Weizmann Institute and the H. Steinitz Marine Biology Laboratory (Eilat) of the Hebrew University. The main problem would appear to be the harvesting of the alga; possible centrifuge methods are under investigation.

Algae production as a means of fixing solar energy is a popular subject in Israel, which hosted an international conference on the subject at Acco last September. One of the most imaginative schemes, worked out by a team from the Technion, headed by Professor Gedaliahu Shelef, uses photosynthesis, bacterial action and algae growth to purify sewage by a process of aerobic digestion accelerated by aeration and flotation techniques. The algae is recovered and used as high-protein animal feed, and the recovered effluent can be used for irrigation. A pilot plant is already in operation in Haifa; introduction of the process on a commercial scale, says Professor Shelef, could save the Israeli economy the several million dollars a year presently spent on the import of soybeans and fishmeal.

The use of essentially natural processes as energy sources seems to have a particular appeal to Israelis, both from an economic point of view, and also, as was frequently pointed out at the Intecol conference in Jerusalem last September, Israel is geographically too small a country to carry an ecological disaster or major pollution.

One such process, which is now being implemented on a pilot scale in

the Negev, is the solar pond. The original "solar lake" was discovered in 1967 in the Sinai, just south of the former frontier. It is a brackish water lake, with high salinity caused by evaporation, into which, during the winter, a certain quantity of less saline water seeps, forming a top layer. Since the lower layers are so dense, no convective mixing can occur, and accordingly, under the Sun's heat the bottom waters can reach a temperature of up to 60 °C. In an artificial solar pond, a suitable heat exchange system can be installed, and the heat extracted. The Weizmann Institute have even considered the possibility of using the Dead Sea itself as a solar lake. According to their calculations 50 square miles of the Sea would provide enough energy to double Israel's water resources by utilising the heat produced in a desalination process.

Perhaps, however, the most imaginative use of solar power is not as an energy source at all, but as a means of controlling soil-borne pathogens. In a process developed by Professor Ja'acov Katan of the Hebrew University, instead of using pesticides, with all their problems of side-effects on non-target organisms, biodegradation, and the like, the soil to be disinfected is simply covered in the summer by a sheet of plastic.

Field tests, taking samples from three layers of soil, showed that after 13 days the pathogens were killed even in the lowest layers. Block-randomised tests give dramatic evidence of the effectiveness of the method, which has already, incidentally, been adopted by some cotton farmers in the USA to control *fusarium*.

The main cost of the method is the plastic, which deteriorates in the strong sunlight and is therefore not reusable. Moreover, it is easy to apply—all the farmer has to do is to unroll the plastic and anchor it firmly.

It would therefore, says Professor Katan, be an ideal method to introduce in the third world, many countries of which share with Israel the prime requirement of any major solar energy programme—a hot climate and a clear sky. □

Red Sea muds ripe for exploitation

DEEP waters containing hot brine and metalliferous muds have been known to exist in the Red Sea since the mid-1960s. However, exploitation of this mud has been hindered by a mild dispute between Saudi Arabia and Sudan over Sovereignty of the Sea, by a lack of resources and by the absence of a suitable technology for mining it.

In May 1974 Saudi Arabia and Sudan signed an agreement on the joint exploitation of the metalliferous mud.

Under the agreement the Red Sea is divided into three zones: one extending westward from the Saudi coast to a line where the depth of water is continuously one thousand metres; one extending eastward from the Sudanese coast to a line where the depth of water is continuously one thousand metres; and a common zone lying between the two others. Each country has exclusive rights over the area between its coastline and the common zone; in the com-

mon zone both countries have equal and exclusive rights.

The agreement paved the way for exploiting the mineral wealth in the Red Sea. Almost exactly a year after the agreement was signed, the two sides established the Jeddah based Saudi-Sudanese Commission for the Development of Red Sea Resources. Each country is represented on the commission by three members; the head of each delegation being the

ministers of mineral resources. The commission is largely funded by the Saudis who hope to obtain a good return on their investment.

The commission is mainly interested in a 60 sq km area known as the Atlantis II deep. It lies midway between Jeddah and Port Sudan in the central trough of the Red Sea. It is here that the metalliferous muds are found. These muds contain 17 different metals in economic quantities ranging from zinc, silver, copper, cadmium, mercury, gold to a great deal of iron. The commission is undertaking extensive surveys and feasibility studies which are expected to be completed by 1981 at an overall cost of US \$100 million.

Before mining can begin a number of technical problems must be overcome. The Atlantis II deep cannot be mined with existing technology so the commission has had to develop its own technology for mining and processing the muds. The commission has done this with the Bureau de Recherches Géologiques et Minières of France, as technical consultants and with the assistance of the Arabian Geophysical Surveying Company, the Arab League Educational, Cultural and Scientific Organisation, the German mining company Preussag and the Universities of Hamburg and Duke.

Small mountains of the mud, which

consists of a superfine material exist in a highly saline, hot environment. The system developed to mine the mud involves agitating it to dilute it with sea water, and then sucking it up with a hydraulic pump. Special ship-borne flotation cells will collect a 5% concentrate of mud in brine. The concentrate will then be carried off to the shore for further treatment and metal extraction. The method of treatment and separation of the metals is claimed by the commission to be cost-effective with a recovery rate of 95%.

Pilot experiments on mining and the separation of metals have already been carried out and further experiments are planned. The mining system will soon be tested in the North Sea and the Mediterranean before final tests begin in the Red Sea. A Red Sea pilot test is planned for February next year.

Disposing of mining wastes has also presented a serious technical problem. Despite the increase in traffic through the Suez Canal, the Red Sea is one of the cleanest seas in the world. The commission is aware that its activities could be detrimental to the environment and sea life of the Red Sea.

The main environmental hazards will result from the diluted muds pumped up and the chemicals used in the flotation process being dumped back into the sea. It is expected that

the pollution would ultimately drift to those areas of the Red Sea that are rich in marine life. The commission is undertaking an extensive research programme, monitoring the environment and controlling the possible effects of this pollution.

The commission is aware that the mining and processing technology developed for the exploitation of the Red Sea, some aspects of pollution control technology, have been tailor made and may not be applicable elsewhere. Other Third World countries, however, can learn a great deal from the research and development activities of the commission. It has, therefore, established an information and documentation centre on the Red Sea. The centre is intended to be used primarily by Saudi and Sudanese researchers and scholars and as a facility for graduate studies. But it will also be open to researchers working on similar problems in other parts of the Third World.

Production is expected to begin around 1985, the yield being 100 thousand to 150 thousand tons per annum over the next 15 to 20 years. The present day value of located reserves in the Red Sea is US \$2.5-\$3 billion. The Saudis and the Sudanese hope to become almost self-sufficient in the minerals they extract from the Red Sea.

Ziauddin Sardar

Nuclear power reaches the Swedish stage

A LITTLE girl is playing a recorder by a river. A monstrous, jerking man closes in on her. She looks up, smiles. "Hullo", she says, "my name is Maria. Shall we play?" She picks flowers. They throw them into the river. She holds out her hands to him. He picks her up, and throws her into the river.

At the interval, two men approach me in the foyer. Each wears a T-shirt. On one T-shirt I read "nuclear reactor"; on the other, "nuclear weapons". As they go by, I see that they are joined by a tape which reads "nuclear waste".

The drama and the facts of nuclear power: nuclear reactors as Frankensteinian monsters, and nuclear reactors as objects of discussion and debate. This is the subject of the most controversial autumn theatre Stockholm has to offer: "The Storm" ("Stormen").

Originally billed for a nine-night run, but prolonged to more than twice that by public demand, "The Storm" is no ordinary production. Nearly all of the Royal Dramatic Theatre's 350 staff members have had a hand in designing the performance, and their enthusiasm is reflected in the energy-and-the-future exhibitions in the theatre's corridors, as well as in the blinking radiation signs, whirling wind vanes and solar panels sprouting from its stately roof.



Frankenstein: a nuclear reactor on the Stockholm stage.

The audience gathers in the main theatre at the beginning and the end of the evening, to see two hearings. The first of these is being held after an accident at a nuclear reactor. Who should take the responsibility for the accident? Various people are questioned. Their answers have been woven into a script from their organisation's actual official texts. The reactor's security chief insists that no accidents can happen; the man from Asea Atom (constructor of reactors) points out that his company only made the tur-

bine, which worked perfectly and had nothing to do with the accident; the lady from the Radiation Protection Institute insists that her institute is responsible for radiation protection only when the reactors are working properly; the State Power Board man can only repeat how much the country needs nuclear power for its balance of payments. The man in the control room at the time of the accident had not followed his instructions, and he is prepared to admit that he must take some of the blame. The message is clear:

the responsibility is nobody's; there is to be one sacrificial lamb.

The crux of the play comes with the second debate: can nuclear power and democracy co-exist? The hearing continues. More witnesses are called: politicians join technocrats. Arguments fly. Again, the message is clear: the complexity of nuclear technology puts it out of the public's control. Democracy has yielded to parliamentarism: the experts rule. But this can be changed if the people demand it. Technology, they should insist, must be intelligible to those who will be affected by it. The people should stand up and fight for a non-nuclear society.

Between hearings, the audience is escorted in small groups through mazes of corridors, past theatre staff notice boards, up and down old staircases and under backdrop stores to see some of the small plays being simultaneously performed in subterranean rehearsal rooms. The play I saw transported us to Baghdad, where a delegation from the International Atomic Energy Agency providing "atoms for peace" ended up supplying the Iraqis with their own atomic bomb.

Reactions to the play have only one thing in common: passion. "Possibly the Dramatic Theatre's most important cultural contribution ever" proclaims Hannes Alfvén, Nobel laureate and nuclear power opponent. "Monstrous lies—the threat to democracy is not nuclear power, but a cynical exploitation of the people's fear of it", declares Lars Lundgren of the Central Electricity Production Board (CDL). "Two things about the play annoyed me personally", adds Bo Kumlin (CDL). "One was a very anti-science feeling: the idea that science and technology as such are bad for the people. This is a very negative approach, which really has nothing to do with nuclear power. The other was that it purposely equated nuclear power with nuclear weapons, making it very hard for the audience . . . to know whether they were talking about the one or the other."

"We have been criticised for not trying to be objective", explains Jan Oqvist, employed as ideas man for the production. "But when the theatre staff started going to see nuclear reactors for themselves, they were shocked. They could not ignore the contradiction of the heavy security precautions, while the guides kept telling them that it was all perfectly safe and that nothing could go wrong. Then they were infuriated by the attitude of some of the scientists they talked to: 'Science is our preserve—what business have you amateurs got to play with it?' We had to impart our experience. The theatre can never be neutral."

Wendy Barnaby

Rocky road to funding



THOMAS H. JUKES

THE return of rocks from the moon by the Apollo expeditions, at considerable expense, gave the geological sciences a unique and unprecedented opportunity for extraterrestrial research with all its far-reaching implications. Biologists could admire the new findings, and try not to be envious. However, *Nature* reported on 10 August, page 522, that the continued funding of moon rock research had been completely blocked by a subcommittee headed by Senator William Proxmire.

It has seemed for some years that the Senator's reach for scientific matters exceeds his grasp of their significance. However, he is apparently alert to the vote-getting potential of issues including science. In 1974, he was successful in eliminating regulations that were designed to curb the expansion of the "health food" industry into ever-widening horizons of vitamin sales. (Incidentally, it seems strange that "health foods" need such heavy supplementation with vitamins.)

The FDA had proposed to restrain the excessive promotion of megavitamin usage by requiring the proponents thereof to establish its usefulness. The curb would have hit the health food stores in the pocket by reducing sales of high-potency vitamin products. The proposal did not affect vitamin pills of lower, but adequate content, each containing 1.5 times the recommended daily allowance. Any amount of vitamins can be obtained by swallowing large numbers of such pills.

Senator Proxmire led the fight in Congress against the regulations, and he introduced a bill to stop them. The bill was opposed by all major professional groups concerned with nutrition, who opined that the "Proxmire bill" catered to the health food in-

dustry and was contrary to the public welfare. It passed the Senate by a majority of 78 to 11, greatly aided by a spectacular letter-writing campaign that was mounted by the National Health Federation. This commercially based organisation sponsors laetrile and opposes fluoridation. One of Senator Proxmire's arguments was that the recommended daily allowances for vitamins had been set artificially too low by the Food and Nutrition Board of the National Academy of Sciences so as to benefit the food and pharmaceutical industries. He said that these industries had subverted the Board by paying its way. The argument seemed strange, because if the allowances were increased, the pharmaceutical industry would sell more vitamins, and the food industry would add increased amounts to foods.

The president of the Academy wrote Senator Proxmire a seven-page letter explaining that the Board was financed solely by a US Public Health Service grant. The letter was unanswered, and when I asked the Senator why, he told me that it was the typical response of a bureaucrat who had been caught in a conflict of interest.

The newspapers help Senator Proxmire by publishing what he calls his monthly "Golden Fleece" award for wasting tax dollars. The award frequently holds research proposals up to ridicule. This is an old-time headline getter. Years ago, a Secretary of Defense, Mr Charles Wilson, announced that his department did not intend supporting research on the question of why grass was green. Once a congressman made headlines by poking fun at an investigation of the "lovelife of cockroaches". This was good for a laugh, because it was well before the search started for pheromones to be used as replacements for insecticides.

Last month Mr Proxmire's target was a \$5,000 expenditure to exercise pregnant pigs on a treadmill. His witty comment on this, as reported by Reuters, was "The folks at U.S.D.A. went hog wild on this one. As a result, the taxpayers may get stuck with a pig in a poke."

To a taxpayer, the palatial Russell and Dirksen Buildings seem at first glance to be sufficiently commodious for the U.S. Senate. Better things are planned: a new, marble, \$135 million palace from which vantage point Senator Proxmire, if re-elected, will doubtless dispense more pearls of wisdom. Perhaps he will bestow a Golden Fleece award on the US Senate.

news and views

Incipient superfluidity in normal liquid ^3He

from P. V. E. McClintock

EXPERIMENTS by D. N. Paulson and J. C. Wheatley, described in a recent issue of *Physical Review Letters* (41, 561; 1978) provide the strongest evidence yet that liquid ^3He anticipates its superfluid transition. It seems that, when the liquid is cooled towards the transition temperature T_0 , its properties first start to deviate from the behaviour characteristic of the normal (non-superfluid) liquid at a temperature which is significantly above T_0 , almost as though the liquid somehow 'knows' that it is about to become superfluid.

At sufficiently low temperatures liquid ^3He does not support ordinary compressional soundwaves because the collision mean-free-path of the quasi-particles which would carry the wave becomes longer than the wavelength of the sound itself: instead, however, there is a peculiar collisionless form of wave propagation known as zero sound. It is this phenomenon which has been investigated by Paulson and Wheatley. Their experiment amounted, in effect, to a very careful study of zero sound and, in particular, of its attenuation coefficient α_0 close to T_0 . The attenuation coefficient, which is defined by how rapidly the wave decreases in amplitude as it travels through the liquid, is known to depend accurately on the square of the temperature T when T is well above T_0 ; but, at T_0 itself α_0 starts to rise very rapidly, in fact almost discontinuously, as T is further reduced. What Paulson and Wheatley wished to ascertain was whether or not any departures from the T^2 dependence could be detected just above T_0 .

Their experimental cell consisted of a pair of quartz transducers which were used, respectively, to excite and to detect the zero sound, thus enabling measurements to be made of the attenuation which occurred within the 12.7 mm path length of liquid ^3He which separated them. The cell was mounted in a nuclear demagnetisation cryostat capable of achieving and

maintaining the temperatures near 1 mK necessary to induce the superfluid transition (the exact value of T_0 depending on the external pressure applied to the liquid). A particularly important feature of the experimental design, distinguishing it from some earlier zero sound cells, lay in the thermometry arrangements.

A paramagnetic susceptibility thermometer was used but, rather than the conventional compressed cylinder of powdered cerium magnesium nitrate (CMN) crystals, the authors used instead a cylinder of cerium lanthanum magnesium nitrate. By replacing 95% of the magnetic cerium ions by inert lanthanum ions, magnetic interactions

between the cerium ions were greatly decreased. This substantially reduced experimental uncertainties involved in converting from the so-called magnetic temperature T^* (defined on the assumption that the temperature is exactly inversely proportional to the measured susceptibility—which it is not) to the absolute Kelvin temperature T . To a good approximation, $T = T^* + \Delta$ where Δ is the required correction: for pure CMN, Δ is about 0.6 mK; whereas, for the diluted CMN used by Paulson and Wheatley, Δ was found to be 0.1 mK or less.

The importance of an accurate absolute temperature scale arose from the need to look for very small deviations from the T^2 law as T_0 was approached: using pure CMN, a small error in Δ can easily produce the illusion of such deviations by introducing a systematic error in T . For the diluted CMN, on the other hand, Δ was already so small that the possibility of spurious effects of this nature could virtually be ignored.

Some experimental results are shown in Fig. 1. The zero sound attenuation α_0 , divided by T^2 is plotted vertically, and the inverse of the temperature T is plotted horizontally. Both quantities have been normalised in such a way that they take the value unity at T_0 . If there were no deviations from T^2 behaviour above T_0 , the graph would follow the dashed horizontal line until the transition at $T_0/T = 1$ was reached, where it would then start to rise almost vertically. Clearly, the reality is far otherwise: there is a small but quite definite excess attenuation, starting well above T_0 which grows increasingly rapidly as T_0 is approached.

Comparable behaviour has also been seen, although not so clearly, in the static magnetisation (Paulson, Kojima & Wheatley, *Phys. Lett.* 47A, 457; 1974) and in the viscosity (Parpia, *et al. Phys. Rev. Lett.* 40, 565; 1978). In each case, the property seemed to start responding to the transition before T_0 had quite been reached. The 'anomalies' were, however, a good deal smaller than in the case of zero sound, and they were hard to evaluate pre-

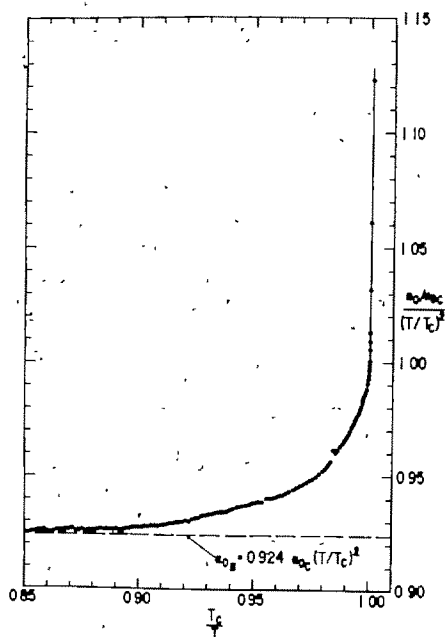


Fig. 1 The attenuation α_0 of zero sound in normal liquid ^3He divided by the square of the absolute temperature T , plotted as a function of T^{-1} . (Both ordinate and abscissa have been normalised in such a way that they are equal to unity at the superfluid transition, where $T = T_0$ and $\alpha = \alpha_0$.) Departures of the experimental points from the horizontal dashed line indicate the extent to which the liquid anticipates its superfluid transition at temperatures above T_0 . (After Paulson & Wheatley, *op. cit.*)

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cisely because of the thermometry problem referred to above.

The reason why liquid ^3He behaves in this way, apparently anticipating its transition, is probably associated with the existence of fluctuations: these may be pictured as the rapid transient formation and disappearance of tiny regions of superfluid, even when the liquid as a whole remains above T_c . In the superfluid just below T_c , α_0 is very much larger than for the normal phase, so that one consequence of such fluctuations might indeed be an 'anomal-

ously' large value of α_0 when T is just above T_c . The magnetisation and viscosity change much less dramatically below T_c , so that the effect of fluctuations on these properties just above T_c would presumably be correspondingly smaller, in agreement with experiment.

There is, as yet, no quantitative theory to describe these phenomena, but it seems probable that strenuous efforts will now be made towards this end—particularly since studies of the corresponding effect in superconducting metals have proved so rewarding.

Conservation in Madagascar

from Alison Jolly

THE first international conference organised by Malagasy scientists on the problems of conserving their unique flora and fauna was held recently in Antananarivo.* The conference was convened to deal specifically with the Malagasy lemurs, a group of primates unique to the island. The government of the Malagasy Democratic Republic has postponed such discussion for the past five years for the good reason that conflict between shifting agriculture and forest conservation is one of the country's most intractable political problems.

Madagascar is one of the world's biogeographic regions. Of its roughly 715 vertebrate species, some 575 are endemic; so are at least 80% of its 10,000 flowering plants. Six families of plants, four of birds, and five of mammals are confined to Madagascar. Intact Malagasy rainforest, deciduous woodland, or spiny desert, where 90% of all the species are unique, show us Hutchinson's "ecological theatre and evolutionary play" with a different cast of characters.

Giving the keynote address, Joseph Andrianampianina, of the Directorate of Water, Forests and Soil Conservation, described lemur hunting, and habitat destruction by axe and fire: slash and burn clearings in the eastern rainforest, forest fires for grazing land in the dry west. When man reached Madagascar little more than a 1,000 years ago, the island-continent was wholly forested. The last full aerial survey, begun in 1949 and published in 1958, showed 20% forest cover. The American states from Maine south through Virginia, with the same area as Madagascar, have almost twice as much forest as Madagascar 20 years ago. The rate of clearance has accelerated to the point where the only

intact forests will soon be those defended by national action. In spite of the scientific importance of Malagasy wildlife, and its possible tourist value, Andrianampianina concluded, "We are perfectly aware that none of our suggestions, none of our practical advice, will achieve anything . . . for the protection of nature, if the people do not have land which they can cultivate in perpetuity in order to live."

Scientific communications at the meeting fell into three groups: morphological and genetic evolution of lemurs, field studies in 'undisturbed' habitat, and papers which took up Andrianampianina's theme of conservation with its social corollaries.

Morphologists seemed to concur, for once, that the Cheirogaleinae, or primitive mouse and dwarf lemurs, form a very distinct group from the Lemurinae or true lemurs like the familiar ringtail. The same picture emerged from multivariate analysis of limb bones (C. Oxnard, J. McArdle, R. German, Chicago; F. K. Jouffroy, Museum Nationale d'Histoire Naturelle, Paris), from the pelvic girdle (C. Berge, MNHN, Paris), the skull (N. Petit-Maire, CNRS, Marseille), and fingerprint analysis (B. Rakotosamamina, Ministry of Higher Education and Scientific Research, Madagascar). This could mean that the Cheirogaleinae will eventually be elevated to family status, which would give Madagascar four primate families of its own, with the Lemuridae, the leaping Indridae, and the Daubentonidae. (Of course, *Daubentonia*, the preposterous aye-aye, may be extinct by then.) Randriamanantenina (University of Antananarivo) presented a new analysis of the subfossil genera of giant lemurs which disappeared about 1,000 years ago with the coming of man. She showed that extinct lemurs were robust in allometric proportion to their size, which suggests that there were no agile brachiators among them. Comparing

limb and vertebral indices confirms Walker's view that *Megaladapis* was a koala-like clinger, but *Paleopropithecus* may have hung upside down like a sloth. H. Hemmer (Mainz) discussed brain size in *Lemur* and W. Padushka (Vienna) the evolution of primate scent marking from ancestors like the Malagasy insectivores.

Chromosome and serum transferrin studies indicate that both *Lemur* and *Lepilemur* are in the course of active speciation (J. Buettner-Janusch, New York University; Y. Rumpel, Strasbourg; J. Andrianivo, Hôpital Gerard et Robic, Madagascar). Each is highly polymorphic in chromosome number, with some genetically isolated species such as *Lemur macaco* or *Lepilemur septentrionalis* which are no more different in morphology than the many races or local variants of interbreeding forms. Buettner-Janusch suggests that much of the molecular and chromosomal variation has arisen in the past thousand years, since the Malagasy central plateau was cleared, leaving a ring of semi-isolated lemur populations round the coast. Coulanges (Institut Pasteur, Madagascar) reviewed the literature on lemur disease. Their physiology differs from other primates: they are apparently immune to polio and to falciparum malaria. It is not known what role lemurs have in the transmission of other malaras, or whether the fact that there is no yellow fever on Madagascar is due to lemurs' immunity, to the ecology of the local *Aedes* mosquito species, or to luck.

Field studies dealt with comparative ecology and behaviour of nocturnal lemurs in a highly seasonal western forest (J.-J. Petter, E. Pages, MNHN, Brunoy), with separation of lemur, bird and insect calls by time or pitch such that each species has its own 'acoustic niche' (L. McGeorge, Duke University, North Carolina), and with foraging strategies in *Indri* (J. Pollock, University of Cambridge). Pollock's indri even draped themselves on a branch and sang their air-raid siren territorial wails in front of forty conference members, which should infuriate all naturalists who have spent days hunting indri in vain. The field work for all those studies, however, was done before the Malagasy Republic suspended research visas for foreigners in 1974. Recently M. Razarihelisoa, R. Albignac, and their students at Antananarivo University have embarked on a study of *Avahi*, the woolly lemur. *Avahi* is nocturnal, lives in pairs in well-defined territories, and eats only leaves. To judge by the conference field trip its movements are as predictable as the

*International Colloquium on Malagasy lemurs, held at Antananarivo, Democratic Republic of Madagascar, on 4-14 September under the sponsorship of the Malagasy Academy.

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teddy-bear it resembles. The study site is an effectively protected forest, a 6-hectare remnant inside the plantation of a paper mill, which has helicopters for fire-fighting. That tiny *Avahi* population may survive, just as white sifaka and ringtailed lemur populations have remained stable since 1963 in the privately protected 100-hectare reserve at Berenty. Lemurs are relatively small and sedentary mammals, so it may be that the minimum critical size of an effective lemur reserve is modest in terms of each species. Larger scale reserves, are needed, instead, to save a representative sample of all the forest types, from rainforest to the thorn-studded, arid, *Euphorbia* forest of the south.

As for direct conservation action, Antananarivo University has just founded a field station with a reserve fenced and guarded on the Berenty model. This is a cooperative project with Yale and Washington (St. Louis) Universities, financed through the World Wildlife Fund and set up by Guy Ramanantsoa (Antananarivo), the conference organiser. After considerable difficulty in finding a local commune which would permit a reserve on its land, he at last chose a stretch of gallery forest in the south, where the local commune welcomed university involvement. This 100-hectare field laboratory is particularly important because it has adequate funds. It is, of course, trivial in scale compared to the 470,000 hectares of designated wilderness reserve which the government has no funds to protect. When Bruno Ratovomboahangy, one of Ramanantsoa's students, described the plight of the rare black sifaka, a race clearly doomed to extinction because its last shred of forest is being cleared, J. Manambolona (Antananarivo), the session's chairman, pointed round the room to four government officials and declared, "This is exactly why we are met, and this is what you must cure!"

Rabe Rafael, Director of Water, Forest and Soil Conservation, presented a detailed proposal for protection of the 580,000 ha of national parks and reserves. He asks for funds amounting to £1.2 million. Less than half that sum would be for the strict forestry needs of personnel and equipment. The other half would go toward formulation of land-use plans and to developing agricultural alternatives for people living around each reserve.

There is now increasing international awareness that the social and economic benefits of conservation are inextricably involved with any park plan. If UNEP, the IUCN, and other international bodies aid ecologically rational development in Madagascar, they could help the Malagasy Republic to save a unique portion of the world's scientific

heritage. If not, the Malagasy will conclude that rich countries only find money to fly their own scientists to conferences. Western knowledge will seem as partial as the expertise of "the man who wears a loincloth that is falling to pieces—he knows exactly how to put it on and how to take it off."

And if nothing is done, the peasants who now roll back the forest will have the same problems in 10 years' time that they do today: eroding watersheds, sterile soil, dwindling firewood supplies. The only answer then will be planted pines or eucalyptus. The option of saving the Malagasy forest communities and hundreds or even thousands of Malagasy species will be gone. □

Halophilic ribosomes

from Richard Brimacombe

COMPARISON of ribosomes from different organisms provides a promising approach to the problem of understanding ribosome structure and function. Several laboratories are engaged on amino acid and nucleic acid sequencing of ribosomal components and are gradually piecing together the information to make such comparisons. One example is recent work on the ribosomal proteins of the extreme halophilic bacterium *Halobacterium cutirubrum* which has come up with some intriguing results. Matheson *et al.* (in *Halophilic Microorganisms* (ed. Caplan) Elsevier/North Holland Biomedical Press (in the press)) have compared the amino acid sequences of the acidic ribosomal proteins—the A proteins—from a variety of halophiles ranging from moderate to extreme. These A proteins are those that seem to correspond with proteins L7/L12 in *Escherichia coli*.

The ribosome of *H. cutirubrum* has been of interest for some years. Whereas the RNA and protein components are similar in size and number to those of *E. coli*, the proteins are very much more acidic (Bayley & Kushner *J. molec. Biol.* 9, 654; 1964). It follows that two-dimensional gel electrophoresis gives little clue to which *H. cutirubrum* proteins correspond to which *E. coli* proteins, and the two organisms are sufficiently unrelated that comparison of N-terminal amino acid sequences is also not very helpful. So Matheson *et al.* have restricted their comparative study to the proteins apparently equivalent to *E. coli* L7/L12 (L20 in *H. cutirubrum*), and to those proteins which bind to

5S RNA. *E. coli* proteins L7 and L12 differ only in the presence of an N-terminal acetyl group on L7, they are distinguished by being the most acidic proteins in the 70S ribosome, and the only proteins present in multiple copies (three to four per 70S). These proteins are functionally important, notably in GTP-dependent processes, and are interchangeable between ribosomes from unrelated organisms such as *E. coli* and yeast (for review see Möller in *'Ribosomes'*, Cold Spring Harbor Press, 711, 1974).

The complete amino acid sequence of *E. coli* L12 (EL12) has been known for some time (Terhorst *et al. Eur. J. Biochem.* 34, 138; 1973), and Matheson *et al.* find that, whereas the amino acid sequences of the equivalent proteins from moderate halophiles are very similar to that of EL12, the sequence of L20 from *H. cutirubrum* (HL20) (which is now complete except for an unordered highly acidic region between residues 78 and 106) has some very interesting similarities and differences. A simple comparison of the amino acid compositions shows that in HL20 the lysine content has dropped drastically from 13 residues to 1, and there is a corresponding increase of 7 to 22 in aspartic acid residues. 60% of the protein is in fact composed of aspartic acid, glutamic acid and alanine. Further, methyl lysine (present in EL12) is absent, and the valine content is reduced, whereas at the same time two tyrosine residues (absent in EL12) make an appearance. Nevertheless, despite these very large differences, there are still distinct regions of homology between the *E. coli* L12 and *H. cutirubrum* L20 sequences.

The first of these is a region of about 18 amino acids near the N-terminus of HL20 (positions 12–30), which shows a high degree of similarity to EL12, not in the N-terminal region of the latter, but in the middle of the sequence (positions 46–62). Another characteristic region, which is very rich in alanine, is observed in the *H. cutirubrum* protein (positions 57–67, with nine alanines). This region is C-terminal with respect to the homologous region just mentioned, whereas the corresponding alanine-rich region in EL12 (positions 34–42, with eight alanines) is on the N-terminal side. Thus the two homologous regions are inverted, and the whole N-terminal region of the *E. coli* protein seems to have completely vanished from the *H. cutirubrum* protein. In addition the basic area of EL12 (positions 57–84) has disappeared in HL20, the latter in turn showing a highly acidic C-terminal region (positions 70–112) which has no equivalent in EL12.

The sequence of *H. cutirubrum* L20

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shows less homology with the moderate halophiles than it does with *E. coli*, suggesting that the extreme halophile has not evolved from the moderate halophiles. Indeed a comparison with the N-terminal sequences of the corresponding N-terminal sequences from two eukaryotic organisms *Artemisia salina* and *Saccharomyces cerevisiae* (Amons *et al.* *FEBS Lett.* **81**, 308; 1977) shows a high degree of homology with HL20 in the first 11 residues, including the two tyrosine residues mentioned above. It seems therefore that the extreme halophile has both pro and eukaryotic character, an idea which is supported by the studies on the N-terminal sequences of the 5S RNA-binding proteins reported by Matheson *et al.* as well as by the sequence of the *H. cutirubrum* 5S RNA itself. Matheson *et al.* draw attention to the recent work of Magrum *et al.* (*J. molec. Evol.* **11**, 1; 1978), which places the extreme halophiles in the Archaeobacteria, primitive organisms which are little related to either pro or eukaryotes.

The amino acid sequence data are interesting in themselves, but there is another aspect of the *H. cutirubrum* ribosome which is equally important in

comparative studies, namely the halophilic property itself. *H. cutirubrum* ribosomes require at least 3.4 M KCl and 100 mM magnesium for stability, a condition under which 'normal' (that is *E. coli*) ribosomes would lose a substantial amount of their protein. Conversely, under the low salt conditions used for protein synthesis with *E. coli* ribosomes, the *H. cutirubrum* ribosome loses much of its protein (Bayley *J. molec. Biol.* **15**, 420; 1966). L7/L12-type proteins have a very high α -helix content, and have alanine-rich sequences similar to those in motile proteins (Amons *et al.* *FEBS Lett.* **86**, 282; 1978). In the case of HL20, the instability of the ribosome at low salt concentration can be at least partly related to this factor, since Matheson *et al.* find that HL20, unlike EL12, loses most of its helicity at salt concentrations below 2 M.

Thus, *H. cutirubrum* L20 is a protein which is part eukaryotic, part prokaryotic; the prokaryotic region of the amino acid sequence is back-to-front, and the protein has to function in a ribosome whose salt requirements are upside-down. The system offers fascinating possibilities for comparative studies of structure and function. □

Plant cells without walls?

from Jeremy Burgess

ISOLATED plant protoplasts are emerging slowly from their former state as an interesting curiosity and a subject for speculation to a new level of usefulness as a research tool, with their main application in virology and cell wall studies. Protoplasts are particularly valuable to the plant virologist as synchronous infection of a high percentage of a population can be easily achieved. This allows the study of the sequence of events as an infection develops in a way which is all but impossible using whole plants. For cell wall studies, protoplasts offer a rare chance of access to a naked plant cell plasma membrane which will, in some species at least, produce a functioning wall structure after only hours or days of culture.

One assumption underlies all this work on protoplasts, and it is now being brought into question. This is that a protoplast behaves as a plant cell without a wall. Mechanically this definition is certainly correct, but is it accurate in a biological sense? If it is not, and results obtained with protoplasts cannot be applied to whole plants, then the usefulness of the

protoplast as a model system is undermined.

The problem arises out of the way in which protoplasts are prepared. In brief, a tissue such as a leaf is first immersed in a concentrated solution of a sugar to which the plasma membrane is impermeable. This causes the protoplast to shrink away from the wall which bounds it, as water is withdrawn by osmosis. Once plasmolysis has occurred, the wall is enzymatically digested away.

The protoplasts are released into suspension as individual units stabilised against bursting by the sugar solute. So during its preparation the protoplast has been partially dehydrated; the connections between adjacent cells in the tissue, and between each cell plasma membrane and its surrounding wall have been severed and the cell wall has been removed. Naturally it was the third of these processes which concerned early workers with protoplasts. Now it seems that not enough attention has been paid to the effects of the other two.

Several recent papers have cast doubts on the assumption that freshly isolated protoplasts behave simply as plant cells without walls. Ruesink (*Protoplastes et fusion de Cellules*

Somatiques Vegetales, 41, CNRS, 1973) has shown that leucine uptake by plasmolysed protoplasts is drastically inhibited compared with intact cells. Racusen, Kinnersley and Galston (*Science* **198**, 405; 1977) examined electrical potentials in normal and plasmolysed tobacco leaves as well as protoplasts. They were able to show that plasmolysis results in the normal negative potential within the cell being converted into a small positive one. If the protoplasts were cultured for 10-14 days, those which reformed a cell wall also regained the negative potential. By contrast, protoplasts of maize which failed to produce a functional cell wall in culture also failed to regain the normal negative potential (Kinnersley, Racusen & Galston *Planta* **139**, 155; 1978). Changes in levels of protein synthesis and photosynthetic rates also occur in plasmolysed cells. These effects of plasmolysis on the general vitality of protoplasts are not immediately reversed. Premecz *et al.* (*Plant Sci. Lett.* **9**, 195; 1977) used RNase levels as a measure of response to stress and found that the concentration of plasmolysing solution used affected the time taken for the recovery of normal physiology. It seems clear that the initial plasmolysis and the subsequent culture in a concentrated sugar solution both present the protoplast with a stress which alters much of its biochemical behaviour compared with the normal plant cell. Thus freshly isolated protoplasts should not be regarded as plant cells without walls, but as a stressed system requiring a recovery period. For example, we have shown the need for such recovery period in the case of cell wall growth on tobacco leaf protoplasts (Burgess *et al.* *Planta* **139**, 85; 1978). Although on first isolation an 8-hour lag precedes visible wall formation, subsequent removal of this wall without imposing additional osmotic stress on the protoplasts allows a new wall to begin to form within 12 minutes.

There is no doubt that in the long term (a period measured in days rather than hours), protoplasts from many sources can completely overcome the effects of osmotic stress. Whole plants have been grown from single protoplasts in a handful of species, and many others will regenerate walls and divide, even if the cells subsequently fail to differentiate into organised tissue. However, most of the current interest in protoplasts is centred on the initial minutes or hours following isolation. This is the period when they are amenable to virus infection, when fusion frequencies are at their highest, and when the first cell wall components begin to be secreted across the membrane. That this is a period of high

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stress for the protoplast should not be overlooked. One area of growing interest is the isolation of the plasma membrane to examine its properties with regard to the synthesis of cell wall components, and also its interaction with virus particles. For results from this kind of study to have meaning in the context of the intact plant cell, allowance must be made for the effects of osmotic stress and dehydration. At present there seem to be two ways of minimising these difficulties. The first is to use a second enzyme treatment on protoplasts which have recovered for a few hours in culture. This approach, however, assumes that the protoplasts will recover. The second way might be to ensure that the cell is not so greatly stressed initially. Colman and Mawson (*Z. Pflanzenphysiol.* 86, 331; 1977) have found that gradual plasmolysis of leaf tissue is necessary for the isolation of photosynthetically active cells. Such a refinement in technique applied to protoplast isolation might well increase the range of species which will respond to culture. Any improvement in the physiology of freshly isolated protoplasts is to be warmly welcomed. □

Nucleon-nucleon interactions

from a Correspondent

THE study of the forces between free nucleons, at energies not much greater than their rest masses, falls between the present interests of nuclear structure and of elementary particle physics. Administratively this is awkward and in Britain nucleon physics is neither popular nor lavishly funded. However, since the 'meson factories' in several other countries began operating a few years ago there has been a resurgence in studies of this fundamental interaction, which is now sufficiently well understood that we are on the verge of an accurate and coherent account of the properties of light nuclei, and of the simplest processes of meson production. These facts, which had been hinted at a year ago at the Vancouver Conference on the Nucleon-Nucleon Interaction, emerged clearly at a recent meeting in Graz*.

At all energies up to about 400 MeV proton-proton elastic scattering is accounted for by the exchange of virtual mesons, together with a set of unambiguous and well-determined phase shifts in the S and P partial waves.

The fine details of this phenomenology are due to measurements at small scattering angles by R. Hess's group from the University of Geneva, who have measured a variety of spin-dependent variables at SIN, Switzerland; and from similar measurements, over a wider angular range, performed at TRIUMF, Vancouver, by the BASQUE group (D. V. Bugg *et al.*; Queen Mary College, London; University of Surrey; Universities of British Columbia and Victoria). So well are these data described by boson exchange potentials (as developed, for example, by R. Vinh Mau's group at IPN, Orsay) that, in the opinion of G. H. Thomas (Argonne National Laboratory) no further measurements of this type are required.

Until very recently the corresponding neutron-proton data have been of poor quality, the experiments being more difficult and the available neutron beams less intense. At Graz, new measurements of triple scattering parameters were reported by the BASQUE group (D. V. Bugg *et al.*; and of the differential cross section for charge exchange, by both E. Rössle's University of Freiburg group, working at SIN, and a LASL/Texas A & M/University of Texas team at LAMPF, Los Alamos (B. E. Bonner *et al.*). The remarkable agreement between the two sets of cross sections disposes of the long-outstanding uncertainty about this quantity. The quality of these data, and of the BASQUE measurements, is now as good as the proton-proton data, and illustrate the capabilities of the accelerators at TRIUMF, SIN and LAMPF.

The BASQUE measurements have been incorporated in a number of partial wave analyses, and as R. A. Bryan (Texas A & M) showed, not only are the isosinglet phases now unique below 400 MeV, but the theoretical interpretation has altered significantly in the past year to give excellent agreement with the new data. This is seen, not only in the work of Vinh Mau's group, presented at Graz, but also in some recently-published work by the University of Florida group (Veda & Green *Phys. Rev. C* 18 337; 1978). Likewise, near 50 MeV, a careful set of measurements by F. P. Brady *et al.* (University of California, Davis) has clearly exposed the incorrectness of earlier data, which had previously been an embarrassment to the theoreticians.

With such excellent understanding of elastic nucleon-nucleon scattering, the way is now open to incorporate these theoretical models into the potentials used in nuclear structure calculations. This was made clear in a lucid review by K. Hollinde (Bonn

University). Incidentally, the perennial fear that off-mass-shell effects may dominate such many-body systems seems groundless; the considerable effort devoted to nucleon-nucleon bremsstrahlung has failed to turn up any evidence for gross departures from elastic amplitudes. Though off-mass-shell effects exist (they are needed, for example, to account for the cross-section for the capture of thermal neutrons on hydrogen), they are clearly weak at the energies considered here.

At higher energies, uncertainties multiply. Are there resonances in one or more partial waves, for example the $^3F_2$ and $^1D_2$, as N. Hoshizaki (Kyoto University) has suggested in a recent preprint? The phase-shift analyses are ambiguous above the inelastic threshold. Using a coupled channel approach, E. Lomon (Massachusetts Institute of Technology) was able to relate the BASQUE data below 500 MeV to possible resonances above this energy. Di-baryon resonances can appear in quark models, and this was discussed in several contributions, such as those of J. J. de Swart (Nijmegen) and C. de Tar (MIT). What is needed are better experimental data on spin-dependent amplitudes near 1 GeV, and so far only the ZGS at Argonne has provided this. The untimely shutdown of the ZGS next year will leave Saturne II, the revamped accelerator at Saclay, alone in this field, and the nucleon-nucleon programme there, under F. Lehar, should be of great interest.

The key to our understanding lies in the inelastic processes. J. A. Niskanen (University of Helsinki) has developed a model of pion production in p-p scattering which goes well beyond the simple Mandelstam model of 25 years' standing, by incorporating explicit isobar components into the nucleon configuration. G. Jones (University of British Columbia) reported on measurements from TRIUMF which were in strikingly poor agreement with Niskanen's model. But Mandelstam's model itself is inherently unsatisfactory; it predicts that pion production in n-p collisions is due only to the isovector part of the amplitude. E. Rössle's group presented data taken at SIN which seem to contradict this, though the experiments, and their interpretation, are difficult. Their results, and those of Jones, also show compelling evidence for d-wave pion production, even very close to threshold.

If the scattering states of two nucleons are so well understood, why is the admixture of D-state in the bound system, the deuteron, so poorly known? The answer is that the D-state arises from a tensor coupling, which is due to the exchange of rather heavy

*The 8th International Conference on Few Body Systems and Nuclear Forces was held on 24-31, August in Graz, Austria.

mesons which evoke short range forces; and the interaction at small distances (≤ 0.8 fm) is not well understood. M. Fiedeldey (Pretoria) showed that several ingenious suggestions for enhancing the experimental sensitivity to the D-state were impracticable, though D. Roger *et al.* (CEN, Saclay) showed that the quasi-free electron scattering experiments now command the accuracy needed for the task. It remains a tantalising problem. Neutron-deuteron backward scatter-

ing was studied by Bonner's and Rössle's groups at Los Alamos and SIN. Their data again agreed well, and the interest here was in a departure of the cross section from a smooth exponential near 180° . This unexpected behaviour occurs between 380 and 650 MeV. No doubt by the next Conference in this series, scheduled for 1980 at Eugene, Oregon, explanations will have been proposed; new puzzles will also have arisen in this exciting, and exacting, field. □

Rats, pigs and men in New Guinea

from Robert M. May

MAN'S impact on the New Guinea highlands dates back five or six thousand years, when widespread development of a shifting slash and burn agriculture led to substantial forest clearance, and, at the same time, the domestic pig was introduced (Bulmer *J. Soc. Océan.* 31, 7; 1975). Over the subsequent millennia, patterns of sedentary settlement gradually emerged. Major disturbance of habitats is, however, probably relatively recent, stemming from the introduction of the sweet potato (*Ipomoea batatas*) as a major food crop some 300 to 400 years ago; this innovation led to increases in population density and to the development of intensive pig-keeping at higher altitudes.

The most conspicuous change wrought by human activities in the New Guinea highlands is the replacement of forest by grassland, maintained by intermittent burning. These increasing areas of man-made grassland exist in a flux of various successional stages, as old gardens or village areas are abandoned and new ones created. The associated long-term changes in the distribution of animal species are shown by recent archaeological studies. One revealing study is from a site at Aibura, in the Eastern Highland Division, where it can be seen that over the past 1,000 years the forest-dwelling phalangerines (for example, *Phalanger* or cuscus species) have declined while the grassland pademelon (*Thylogale*, a relative of the rock-wallaby) has concomitantly increased (White, *Ol Tumbuna: Terra Australis* No. 2, Australian National University, 1972).

A particularly detailed account of the present-day distribution patterns of seven species of terrestrial rats in relation to the changing habitat in the New Guinea highlands has recently been given by Dwyer (*Aust. J. Ecol.* 3,

213; 1978). The seven species all belong to the Old World family of murid rodents, four from the genus *Rattus* and three from *Melomys*, as tabulated below. The 'productivity index' is a measure of the intrinsic capacity for population growth of the species, which Dwyer computes by multiplying the mean litter size by the average proportion of females (above minimum adult weight) that are in breeding condition. By thus using the proportion of adults that are breeding, Dwyer takes some account both of the number of litters per year and of the age structure; the quantity is, moreover, weighted to allow for differences between wet and dry seasons.

Species	Productivity index
<i>R. exulans</i>	2.10
<i>R. ruber</i>	1.74
<i>M. rufescens</i>	1.18
<i>R. verecundus</i>	0.94
<i>M. levipes</i>	0.60
<i>R. niobe</i>	0.42
<i>M. rubex</i>	0.35

As a very crude generalisation, we might expect the successful species in the relatively more disturbed and unpredictable grassland habitat to tend towards the 'r-strategy' of "high reproductive rates, short life spans, large population size, . . . and broad, often cosmopolitan distribution" (Grassle & Saunders *Deep Sea Res.* 20, 643; 1973), while the successful species in the relatively undisturbed and predictable forest habitat may tend towards the opposite 'K-strategy' (for more full exposition of these notions, see *Nature* 257, 737; 1975; 267, 394; 1977). Indeed Felming (*Ecology* 55, 555; 1974) has interpreted the life history strategies of two species of heteromyid rodents in Costa Rican rainforests in this light: *Liomys salvini* in the seasonal highland

forest as an r-strategist and *Heteromys desmarestianus* in the season-free lowland forest as a K-strategist. Similarly Freeland (thesis, Queensland University, 1972) has suggested that two murids in a subtropical Eastern Australian rainforest coexist by virtue of *Rattus fuscipes* exploiting an r-strategy keyed to environmental features that are unpredictable while *Melomys cervinipes* is adapted to relatively predictable features. If these simple ideas are to apply to Dwyer's more complicated seven-species system, we expect the habitats to manifest a decreasing degree of disturbance as we run down the species list in the table above; that is, we expect the 'productivity index' to be significantly larger in more disturbed habitats.

Dwyer shows this is indeed so. His extensive trapping studies in the Mt Erimbari-Mt Homo region at the western boundary of the Eastern Highland Division of Papua New Guinea show that the short grass communities (which are created and sustained by man) support only *R. exulans*. Notably, this is the only species on the list that also lives in houses. *Miscanthus* communities represent a later successional stage that is still short grass, and they are also largely under human control; here are found *R. ruber* and *M. rufescens*. *R. verecundus* and *M. levipes* are found in the extensively disturbed oak-dominated forests of Mt Homo, and *R. verecundus* and *M. rubex* occur together in the lower and more disturbed forests of Mt Erimbari; *R. verecundus* is more tolerant of areas regularly disturbed by pigs. High on Mt Erimbari, *M. rubex* and *R. niobe* are found together where disturbance by humans and pigs is least. The few trappings of *R. niobe* below 2,400 m were from well-protected sites. *R. niobe*, in particular, seems to need dense cover of the kind that is incompatible with piggly attention; the existence of this species elsewhere in high (and undisturbed) grassland and in forests at lower altitudes shows that neither altitude nor vegetation alone confine it within the narrow bounds observed in Dwyer's study area. Other aspects of the distributional ecology also fit the scheme: the density of *R. exulans* in grassland was 20 per ha, and of *R. niobe* and *M. rubex* on an equal grid in mixed forest were 8.0 and 4.6 respectively; the mean monthly survival probability for *R. exulans* in grassland is around 0.77, and for *R. niobe* and *M. rubex* in mixed forest it is 0.90 and 0.92 respectively. In short, these gross patterns of distribution correlate remarkably well with the 'productive index' tabulated above. Insofar as there are discrepancies, they are that for a ranking based on habitat, *R. verecundus* might rank below *M.*

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levipes, and the reclusive *R. niobe* below *M. rubex*; these departures are easily rationalised by appeal to differences between genera (for example, each *Melomys* is larger than its *Rattus* partner)

Additional support for the idea that the distribution patterns are governed by the amount of disturbance by man and pigs comes from the archaeological record. White's (*op. cit.*) excavations have shown a marked increase in the representation of small rodents in the upper horizons of his site. Tooth measurements suggest that the three species present are *R. niobe*, *R. exulans* and *R. ruber*; of these, the first is found only in lower levels, the second only in the top level, while the third increases over time in step with increasing signs of human activity.

Dwyer concludes "that the local diversity of small terrestrial rodents in rain forests of densely populated areas of the New Guinea highlands was higher in the past than it is today and that reduction has been a consequence of habitat disturbance by men and pigs producing a smaller, less continuous, area of rainforest and simplification of ground cover. The pattern of local species distributions is now dominated by gross disturbance effects with the more opportunistic of rainforest species found in the most disturbed rainforests. Some species may have dropped out of local faunas" He also suggests that some aspects of the distribution patterns of the montane avifauna of New Guinea (as extensively studied by Diamond and others) may be associated with recent disturbances rather than with 'long term' 'island equilibrium' effects. In common with many other workers, Dwyer sees disturbance as important in promoting and maintaining species diversity, essentially because it enlarges the number of different roles in the evolutionary play. But severe disturbance, of a kind outside the evolutionary experience of the ecosystem, will make for less diversity.

The asthenosphere as point defect zone

from Peter J. Smith

At depths in the Earth of somewhere between 75 km and 250 km there is a layer perhaps some 75–150 km thick in which seismic velocity is slightly lower, and seismic attenuation is rather higher, than in the regions immediately above and below. Any uncertainty implicit in this statement arises because the depth and thickness of the layer, which may in any case be variable,

have not been determined everywhere and because, even where experiments have been carried out, the boundaries of the layer are often diffuse. Nevertheless, there seems little doubt that this 'low velocity layer' exists in some form beneath most of the Earth's surface. Moreover, the layer is now usually equated with the asthenosphere, the semi-mobile zone which allows the rigid tectonic plates of the overlying lithosphere to move.

But accepting the identity of the asthenosphere and the low velocity layer, what is the physical cause of the mobility, the lower velocity and the higher attenuation? The majority answer is partial melting of peridotite. Suggestions about the nature of the cavities in which the melt fraction supposedly resides have included spherical pores, coin-shaped cracks and tubular channels; and estimates of the amount of partial melting accordingly required to account for the seismic observations have ranged from about 6% to 0.1%. Whatever the cavity shape, the degree of partial melting required is apparently low; and so if the low velocity zone were purely a static phenomenon there would be little to choose between models.

The reality is more complicated, however, for the molten material can evidently flow, which means that in at least some parts of the asthenosphere the melt cavities must be interconnected. And that seems to pose quite a problem. For according to Shaw (*Geophys. Res. Lett.* 5, 629; 1978), Walker *et al.* (in press) have investigated the stability under gravity of a melt–solid system with interconnected porosity and have thus shown that for tubular channels, instability will arise if the melt fraction exceeds 0.1% (which compares with the roughly 1% required to explain the seismic observations). Above 0.1% the melt segregates and rises from the solid in less than 10⁷ yr. Walker and his colleagues therefore conclude that the low velocity layer is either a temporary phenomenon which just happens to be well developed at the moment or a "dynamic system with a melt fraction above their calculated limit of long term stability through which melt must flow continually at a substantial rate, in fact, at a rate which is probably unrealistically high".

Of course, many assumptions are involved, any one or more of which may turn out to invalidate the Walker model as a reasonable semblance of reality. Nevertheless, at the very least the results are such as to suggest that partial melting should not be accepted

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too readily, as, indeed, some workers have long realised. Guegnen and Mercier (*Phys. Earth Planet. Interiors* 7, 39; 1973), for example, listed several other possibilities and concluded that the seismic properties of the low velocity layer could in theory be as well explained in terms of viscous grain boundary relaxation or dislocation–impurity interactions.

Unfortunately, there is little experimental data to support either of these particular solid state processes as a cause of the asthenosphere; but Shaw has now added one phenomenon for which there is some empirical (and theoretical) support: namely, the presence of point defects. Shaw himself (*J. Phys. Chem. Solids* 35, 911; 1974) has shown that in the B1 phase of silver iodide, variation in the concentration of lattice point defects leads to a very rapid decrease in sound velocity with increasing temperature and an equally rapid increase with increasing pressure. The magnitudes of defect-produced velocity changes in materials likely to be important in the Earth (such as silicates and oxides) are far less well known and may in any case be different for different types of defect (Frenkel defects, Schottky defects for example). But Shaw has little difficulty in showing that taking into account the probable relative temperature and pressure effects in materials and the variation of temperature and pressure in the Earth, a seismic velocity anomaly in the Earth would be expected in the depth range 50–250 km—precisely where it is observed.

Considering the uncertainties involved in Shaw's theoretical development, this agreement is amazing. And it is all the more encouraging in that defects are known to facilitate material transport, thus enhancing flow and leading to a decrease in apparent viscosity. If Shaw is right in his thinking, the presence of point defects offers just as reasonable an explanation of the low velocity layer–asthenosphere as does partial melting, although to prove the point there will have to be far more experiments or probable Earth materials at the appropriate temperatures and pressures. □

DNA polymerase and mutation

from Bryn Bridges

THE underlying importance of damage to DNA and its mutagenic consequences in the production of cancer and certain heritable diseases becomes steadily more apparent, and it may well extend to such superficially unrelated

diseases as atherosclerosis and certain neurological conditions. The steps by which damaged DNA generates permanently altered nucleotide sequences are, however, obscure in mammalian cells. The question is probably closest to resolution in bacteria where there seems to be an intriguing interaction between DNA damage and the fidelity of DNA polymerases.

Ultraviolet light is commonly used as an experimental DNA damaging agent and initiates mutagenesis through constitutive or inducible pathways depending on the circumstances. The existence of this inducible pathway, proposed and termed 'SOS' repair by Miroslav Radman, is best illustrated by the fact that UV-irradiation of bacteriophages λ or ϕ X174 leads to the production of mutations only if the phages are plated on bacteria that have themselves been UV irradiated or given some other 'inducing' treatment.

A mechanism for the action of DNA polymerases in ultraviolet mutagenesis has now been proposed by Radman's group (Villani *et al. Proc. natn. Acad. Sci. U.S.A.* **75**, 3037; 1978) who have shown that photoproducts produced in the DNA of ϕ X174 by ultraviolet constitute a block to DNA replication by cell-free extracts of *E. coli* operating either through DNA polymerase I or III. (Recent studies have pointed towards the role of DNA polymerase III in bacteria as the mutagenic enzyme, particularly in the inducible pathway (see Bridges & Mottershead *Molec. gen. Genet.* **162**, 35; 1978)). In experiments with purified DNA polymerase I (large fragment) they further showed that DNA synthesis on an irradiated ϕ X174 primed template was accompanied by a large turnover of nucleoside triphosphates into monophosphates, in contrast to synthesis on an unirradiated template.

DNA polymerase I large fragment lacks 5' to 3' exonuclease activity but retains both polymerising and 3' to 5' exonuclease activities. The latter is believed to act as a 'proof-reader', excising newly incorporated terminal bases which are not paired correctly with the template base. Villani *et al.* suggest that ultraviolet photoproducts in the template strand do not prevent incorporation by the polymerase function but that the bases incorporated opposite photoproducts are registered as mismatched and are immediately excised by the proof-reading exonuclease. The net result is that nucleoside triphosphates are converted to monophosphates with no DNA chain elongation, a condition termed polymerase 'idling'. They further suggest that newly-incorporated mismatched

bases could persist and be seen as mutations if SOS induction involved the inhibition of the proof-reading function so that chain elongation could proceed beyond the photoproduct and its potentially mismatched partner. In support of this they give evidence that AMV reverse transcriptase, an error-prone DNA polymerase devoid of any exonuclease activity, is both highly prone to insert wrong bases and replicates UV-irradiated ϕ X174 DNA far above the level of *E. coli* DNA polymerase I.

The findings of Villani *et al.* suggest a possible signalling mechanism for triggering the SOS inducible pathway. SOS mutagenesis in *E. coli* is dependent on the *recA*⁺ gene and it has been thought to be similar in its operation to other *recA*⁺-dependent phenomena such as prophage induction. The *recA*⁺ protein is produced in substantial amounts after ultraviolet irradiation and seems to act in prophage induction by carrying out proteolytic cleavage of the λ repressor. Villani *et al.* suggest that the nucleoside monophosphates produced by idling polymerases may well be the initial inducing signal leading to enhanced production of *recA*⁺ protein and thus to the cleavage of λ and other SOS repressors.

Another, simpler possibility is apparent, however, if we consider the recent work of Byrnes *et al. (Biochemistry* **16**, 3740; 1977) who have shown that nucleoside 5'-monophosphates inhibit specifically the 3' to 5' exonuclease but not the polymerase function of DNA polymerase I, and of Que, Downey and So (*Biochemistry* **17**, 1603; 1978) who have further produced evidence that the nucleoside 5'-monophosphates bind at the primer terminus site for the 3' to 5' exonuclease activity. On the basis of their observations one might argue that SOS mutagenesis induction might be more simply explained as follows. Arrival of a photoproduct at the replication fork causes polymerase III idling and accumulation of nucleoside monophosphates. These in turn directly inhibit the 3' to 5' exonuclease function ('induction') and allow chain elongation to continue with any mismatched bases (both opposite the photoproduct and elsewhere) persisting as mutations. Return to high fidelity replication would occur after normalisation of the nucleoside monophosphate pool. The elementary hypothesis of direct control by nucleoside monophosphate levels suggested here does not, however, have any obvious requirement for repressor cleavage by the *recA*⁺ protein. Although it may seem heretical, perhaps the possibility should be considered that the *recA*⁺ protein does not act proteolytically in ultraviolet mutagenesis but rather interacts directly at the DNA level to

'stabilise' the photoproduct-primer terminus configuration so as to allow polymerase idling. Consequent elevation of nucleoside monophosphate levels would then result in direct inhibition of the 3' to 5' exonuclease function and this in turn would lead to mutagenesis and survival. Certainly a constitutive level of the *recA*⁺ protein exists and the protein is known to bind to single-stranded DNA. Furthermore the γ -irradiation resistance conferred by the *recA*⁺ gene seems to be independent of protein synthesis and does not involve inducibility. Could this also be true for ultraviolet mutagenesis? Such an explanation, while based on slender experimental evidence, would seem to merit consideration, if only because of its simplicity. One would still have to find a role on either hypothesis for other genes (*lexA*, *lexC*, *umuC*, and *recB uvrD*, for example) which affect ultraviolet mutagenesis.

Whether or not the postulated inhibition of the proof-reading exonuclease function by nucleoside monophosphates is direct or indirect will doubtless become clear in the not too distant future. The concept of polymerase idling proposed by Radman's group is, however, very plausible and should stimulate the necessary experiments, particularly those designed to reveal whether it occurs *in vivo* as well as with the purified enzyme fragment. Also to be resolved is the question of whether DNA polymerase III behaves *in vivo* the way the polymerase I fragment behaves *in vitro*, and the question of constitutive mutagenic repair should this, as seems likely, also be mediated by polymerase III. Does this also involve inhibition of the proof-reading function and if so, how?



A hundred years ago

CAPTAIN PATTERSON, Superintendent of the U.S. Coast Survey, has lately initiated a very important undertaking in connection with the work of the Survey, namely, in determining the extent and position of the oyster beds of the Chesapeake Bay, primarily with reference to the formation of oyster reefs, and their interference with navigation, but broad enough in its scope to serve as the basis of a critical investigation of the whole subject of the oyster fisheries and oyster culture in the United States. The work is being prosecuted in the Chesapeake Bay by the Coast Survey Steamer *Palinurus*, Mr. H. J. Rice, formerly of Johns Hopkins University, looking more particularly after the natural history features, such as the embryology and development of the oyster, &c. From *Nature* **18**, 17 October, 653; 1878.

review article

Steroid-induced meiotic division in *Xenopus laevis* oocytes: surface and calcium

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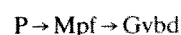
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Progesterone reinitiates meiotic maturation in Xenopus laevis oocytes. Evidence is reported which indicates that the steroid acts at the level of the cell surface and suggests that an induced change of Ca^{2+} distribution triggers in turn a cascade of cytoplasmic events including protein synthesis and germinal vesicle (nucleus) breakdown. These novel features of steroid hormone action in amphibian oocytes are discussed in relation to presently accepted views of the mechanism of action of steroid hormones in somatic cells.

IN TARGET cells, hormone-receptor complexes interact with an effector structure according to two major patterns. The first one, for polypeptide hormones and neuromediators, involves interaction at the plasma membrane level influencing membrane enzyme(s) (for example, adenylate cyclase) and/or cation transfer system(s). In the other case, steroid and thyroid hormone-receptor complexes interact in the cell nucleus with elements of the gene expression machinery as yet poorly defined, and probably modify transcription. We propose here that progesterone action on *Xenopus laevis* oocytes is initiated at the surface level of these germ cells. The effects differ in many important respects from what has been described in steroid hormone target tissues, and they can be mimicked or inhibited by agents which operate at the membrane level and/or induce changes of intracellular Ca^{2+} distribution.

Fully grown *Xenopus laevis* oocytes are arrested at the prophase of the first meiotic division; at this stage 'VI' (ref. 1), they have a diameter of about 1.2 mm, vitellogenesis is completed and the large nucleus (500 μ m) contains replicated DNA. They can be obtained free of follicle cells by appropriate collagenase treatment and can be cultured at room temperature. Progesterone (P) added to the medium induces 'meiotic maturation' *in vitro*²⁻⁴, which proceeds to the metaphase of the second meiotic division. The effects produced by the steroid include the breakdown of the nucleus or germinal vesicle (Gvbd), chromosome condensation, spindle formation and cytokinesis, with the production of an egg ready to be fertilised and the ejection of a small polar body. These events are associated with an increase of proteins related to meiosis, and eventually fertilisation and early embryonic development⁵⁻⁹; protein synthesis seems to be independent of transcriptional control throughout meiosis⁵⁻¹⁰. The eggs obtained *in vitro* are indistinguishable from those produced *in vivo* after gonadotropin stimulation of follicle cells where progesterone is released for direct delivery to the oocyte (without passing through the blood)¹¹; they are able to support cleavage and development after receiving a transplanted blastula nucleus¹².

With progesterone in the medium at 1 μ M concentration, Gvbd occurs *in vitro* after 5-8 h and is easily identified by the white spot observed on the pigmented animal pole of oocytes. During this period, the cytoplasm acquires a 'maturation promoting factor' (Mpf)^{4,13,14}, which can directly induce Gvbd when injected into an untreated oocyte. These results suggest the following sequence:



Mpf is neither progesterone nor a metabolite of progesterone. Indeed, progesterone does not provoke Gvbd when injected directly into the oocyte. It does not act through a nuclear mechanism, and can induce Mpf in enucleated oocytes and in actinomycin D or ethidium bromide treated oocytes. In cells exposed to cycloheximide, the hormone is ineffective; however, Mpf injected into such oocytes produces its normal effect^{12,15}.

We are concerned here with the mechanisms by which progesterone triggers the formation of Mpf, proteins and eventually Gvbd in the incubated oocytes.

Progesterone concentrations required

When oocytes are exposed to different concentrations of progesterone for 8 h, a dose-effect relationship is observed using Gvbd as response (Fig. 1). The half-maximum effective concentration (ED_{50}) is of the order of 10-100 nM. The dose-response curve depends on the time of progesterone exposure: if one observes Gvbd after a much longer time (24 h), lower concentrations of progesterone are found to be active. The 'real' apparent interaction constant of the hormone with the oocyte is difficult to calculate because Gvbd is a late event far removed from progesterone interaction with the oocyte, and as, according to the hormonal state of the female, oocytes are ready to 'mature' more or less rapidly, then each experiment must have its internal control.

It has been reported that the degree of progesterone stimulation of maturation is not dependent on its molar concentration, but rather on the actual quantity of hormone available, and the

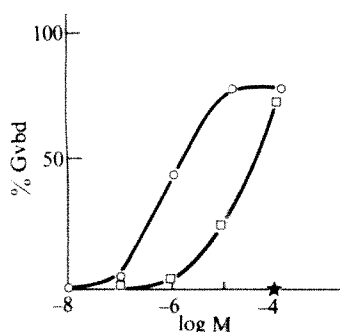


Fig. 1 Progesterone and ACA-poly (EO) activation of meiotic maturation expressed as the % of oocytes undergoing Gvbd. At each experimental point, 100 oocytes were incubated continuously with the inducer (concentrations in incubation medium at zero time), and the % of Gvbd was scored after 16 h exposure. Each point represents the mean of six different experiments on different females. ED_{50} for ACA-poly (EO) (□) and for progesterone (○) were, respectively, 30 and 2 μ M. 0.1 mM O_2 -poly (EO) (★) did not induce maturation.

incubation medium volume for a given concentration¹⁶. However, when enough time is allowed for observing completion of the maturation, the same efficiency is obtained whatever initial volume is used (Fig. 2). Moreover, incubation of oocytes with a polymer-bound steroid (Fig. 3 and below) does not display the apparent volume dependence of progesterone for short incubation periods (Fig. 2); the explanation for this difference may be that the polymer-bound steroid, in contrast to progesterone, cannot enter the cell and subsequently does not undergo trapping and/or transformation to inactive metabolites or metabolites less active than the parent compound.

Steroid specificity of agonists

Many steroids other than progesterone are very active (Table 1). The hormonal specificity is very different from that observed in progesterone-sensitive somatic cells. (This is based on observations in mammalian and avian progesterone target tissues, as we are not aware of similar studies in amphibians.) Thus, testosterone, deoxycorticosterone (acetate) and cortisol are very active. Certain 5 α and 5 β -reduced metabolites of progesterone and testosterone are also active, even though they cannot be metabolised back to the parent hormone. Pre-hormones, which may be transformed into active hormones, are also agonists. However, it must be emphasised that not all steroids are active. Oestradiol is not an agonist, and, as seen on Table 1, only some

of the other oestrene derivatives of the 19-nor-testosterone series are active, and the chemical substitutions which influence activity do not lead to gross differences of charge and solubility. Large nonpolar compounds such as cholesterol and cholestenone, as well as deoxycholic acid, are inactive.

Accordingly, the stereochemical diversity of steroids active in oocytes is impossible to reconcile with the high specificity of steroid hormone-receptor interactions in somatic cells¹⁷. Moreover, the apparent ED_{50} s for the most active steroids are of the order of 10 nM–1 μ M, somewhat higher than the active concentrations of 'free' (non-protein-bound) steroid hormones in the plasma of mammals and also higher than the equilibrium binding constant K_d of steroids with classical proteinic receptors.

No saturation has been observed when studying the physical interaction of active (progesterone) or inactive (oestradiol) steroids with oocytes. This may be related to their large uptake and nonspecific retention by the yolk. These complications account for the absence of publications of competitive binding experiments, whether with agonists or antagonists.

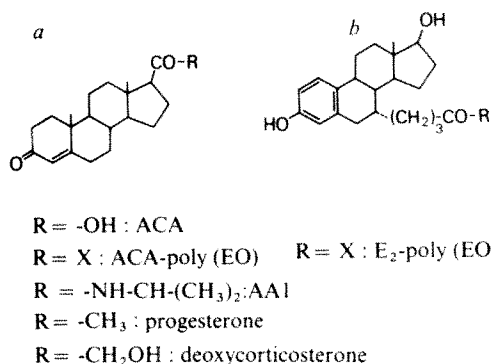


Fig. 3 Proposed structure of the steroid derivatives. $X = -NH-(CH_2)_3-NH-(CH_2-CH_2-O)_n-CH_2-NH-(CH_2)_3-NH-$; ACA = androsta-4-ene-3-one-17- β -carboxylic acid; EO = amino-propyl-polyethylene-oxide. Note that in the case of E_2 -poly(EO), the MW of poly(EO) is 6,000, whereas for ACA-poly(EO), the MW of poly(EO) is 20,000 (see ref. 23).

Despite these difficulties, it has been possible to show that some synthetic steroids which are inactive or weak inducers of meiosis even at very high concentrations, can act as antagonists of progesterone action. Data concerning R-2341, a potent androgen in mammalian tissues, are reported in Fig. 4, and effects of other steroids are shown in Fig. 5. It is also remarkable that we have observed inhibition of progesterone action with oestradiol and other oestrenosteroids (Table 1), but not with diethylstilboestrol, a nonsteroidal oestrogen. Together with the fact that cholane and cholestane derivatives are neither active nor antagonistic, the results give evidence for some type of specific binding site for progesterone action in oocytes which differs profoundly from that known in classical progesterone target tissues.

Site of progesterone action

From the lack of Gvbd after progesterone injection into oocytes, it has already been suggested that progesterone may interact with the surface membrane to exert its effects (refs 13, 18), and no specific binding of progesterone has been found in soluble extract from oocytes. However, microinjected cortisol induces maturation, perhaps due to different physicochemical properties¹⁹. The argument based on the nonactivity of injected hormone for designating the membrane as the site of action is not very strong, as injected Ca^{2+} does not give Gvbd, whereas calcium introduced into the cell by iontophoresis is active.

Fig. 2 Time-dependent induction of maturation in the presence of different initial doses of progesterone or polymer-bound steroid, as expressed as Gvbd. 100 oocytes were incubated in 1 (---) or 10 (—) ml of Barth medium in the presence of ▲, 1 μ M progesterone; □, 0.1 mM progesterone; ●, 0.1 mM polymer-bound steroid (see Fig. 3).

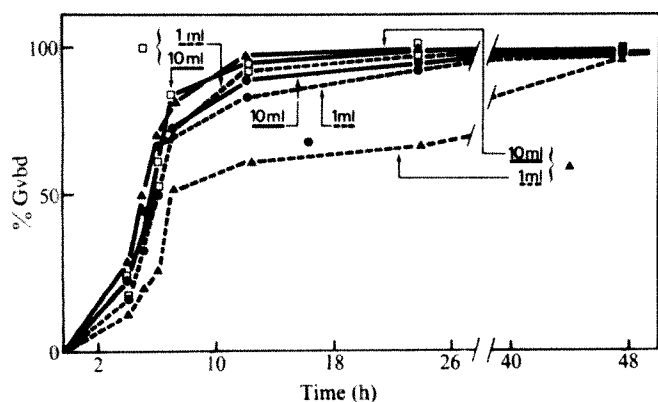


Table 1 Steroids reinitiating meiosis in *Xenopus laevis* oocytes in vitro

Agonists	
Progesterone	The most active natural hormone.
17,21-dimethyl-19-nor-pregna-4,9-diene-3,20-dione (R-5020)	A synthetic progestagen highly active in mammals.
Deoxycorticosterone (and acetate), cortisol, testosterone	Hormones nonphysiologically related to maturation.
Δ^3 -pregnenolone, Δ^4 -androstenedione, dehydroepiandrosterone	Prehormones, metabolisable to progesterone or testosterone.
5 α -, 5 β -dihydroprogesterone, pregnandiol, 3 α -hydroxy-5 α -pregnane-11,20-dione* 5 α -, 5 β -dihydrotestosterone, 5 α -androstan-3 α ,17 β -diol	Metabolites of progesterone or testosterone, not metabolisable back to hormones.
Cyproterone	Progesterone derivative, anti-androgen in mammals.
19-nortestosterone†(17 β -hydroxy-oestra-4-ene-3-one)	A synthetic androgen
Non (or weakly) agonists, but antagonists	
16 α -methyl- or ethyl or ethynyl-17 β -hydroxy-oestra-4-ene-3-one, 17 β -hydroxy-oestra-4,9,11-triene-3-one†(R-2341)	Synthetic derivatives of the 19-nortestosterone series.
Oestradiol, 17 α -ethynyl-oestradiol, estradiol-17 β -valerianate, 8-iso-oestradiol	Natural and synthetic oestrogens
Neither agonists nor antagonists	
13 β -propyl-cortisol, 13 β -ethyl-oestra-4,9-diene-3,17-dione, 17 β -hydroxy-13 β -ethyl-oestra-4,9,11-triene-3-one-17 α -ethynyl (R-2323)	Synthetic derivatives with a 2C or 3C-13 β side-chain (anti-progesterone in mammals).
Diethylstilboestrol	Synthetic nonsteroidal oestrogen.
Cholesterol, cholesta-4-ene-3-one, deoxycholic acid	C27 and C25 compounds.

* Used as an anaesthetic.

† Activities also described in ref. 65.

Melanosomes, however, have been shown to display a selective affinity for active steroids²⁰. This observation is difficult to interpret and cannot be considered to be due to melanin²¹, as oocytes of albino mutants also respond to progesterone.

Our experiments^{22,23} support the hypothesis that the decisive site of progesterone-oocyte interaction is at the surface level. Thus, it has been shown that a progesterone analogue, when linked to a macromolecule, is able to promote maturation without significant entry into the cell. The free steroid (androsta-4-ene-3-one-17 β -carboxylic acid, ACA in Fig. 3) is approximately as active as progesterone on oocytes. When it was linked to a soluble polymer (aminopropylamino-poly(ethylene oxide) poly (EO) of MW 20,000) the complex was found to provoke meiotic maturation with an ED₅₀ of approximately 3 μ M in 16-h incubation experiments (Fig. 1). All effects obtained with progesterone on protein synthesis and Mpf formation were also observed with the steroid-polymer.

Fig. 4 R-2341 inhibition of the progesterone-dependent release of meiosis in the oocytes of *Xenopus laevis*. The data represent the % of Gvbd of 100 oocytes incubated at room temperature in 10 ml Barth medium (pH 7.4). The oocytes are all taken from the same female for each type of experiment (a, b). Maturation was scored after 30 h continuous exposure to progesterone. R-2341 was added to the oocytes 16 h before progesterone. a, Frequency of oocyte maturation induced by 50 nM progesterone as a function of R-2341 concentration. b, Effects of progesterone on the frequency of oocyte maturation in the absence (■) or presence (●) of 2.5 μ M R-2341.

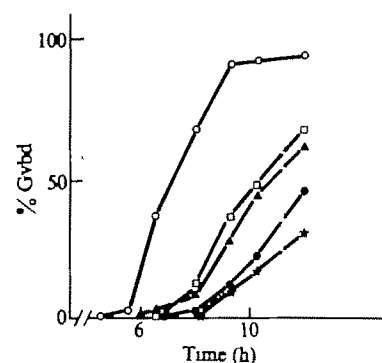
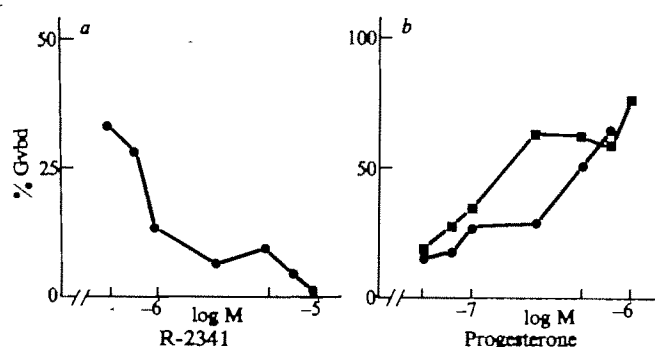


Fig. 5 Inhibition by different steroids of the progesterone-dependent release of meiosis in the oocytes of *Xenopus laevis*. The data represent the % of Gvbd, of 100 oocytes incubated at room temperature in 10 ml Barth medium (pH 7.4) plotted as a function of time after exposure to progesterone 1 μ M (○). The oocytes were all taken from the same female and preincubated for 16 h with: ethinyloestradiol, 10 μ g ml⁻¹ (□); 16 α -methyl-19-nortestosterone, 10 μ g ml⁻¹ (▲); 16 α -ethynyl-19-nortestosterone, 10 μ g ml⁻¹ (●); R-2341, 10 μ g ml⁻¹ (★).

Oestradiol attached to the polymer (up to 1 mM) and the polymer itself did not provoke Gvbd, confirming the specificity of action of the progesterone macromolecular analogue. The possibility of cleavage of the macromolecular derivative with release of active steroids during the incubation was ruled out using radioactive derivatives. These studies showed negligible uptake of the labelled macromolecular progesterone analogue compared to that of free steroids and there was no correlation between uptake and effect on Gvbd. Injection of the polymer-linked progesterone into the oocytes did not induce maturation. Finally, we found that the efficiency of the macromolecular steroid is linearly correlated with the time of actual exposure, in contrast to the curve obtained with progesterone (Fig. 6); this may be due to the hydrosolubility of the derivative, which is easily washed from the surface, contrary to hydrophobic progesterone, which is largely trapped by the oocyte. Concurrent with our studies, induction of meiosis was observed with deoxycorticosterone hemisuccinate coupled to aminoethylated agarose beads²⁴.

These results suggest that the steroid acts at the surface of the oocyte to initiate the meiotic maturation response.

Other inducers at the membrane level

A wide variety of nonhormonal compounds (Table 2), although active at the surface of cells, also trigger meiosis. Among these are several amphiphilic cationic drugs known to penetrate membrane phospholipids and increase the surface pressure.

The best representatives of such compounds are the β -adrenergic antagonists (for example, propranolol). Used in the millimolar concentration range (interaction of propranolol with β -adrenergic receptor has a K_D of approximately 1 nM), they display the so-called 'membrane stabilising effect' or 'local

anaesthetic activity' independently of their stereochemical configuration. It is possible that these effects are related to interaction with membrane phospholipid turnover²⁵⁻²⁹. In contrast, β -adrenergic antagonists lacking the hydrophobicity of propranolol, such as atenolol, practolol and sotalol, are not able to induce meiosis. The two latter compounds have no effects on phospholipid metabolism in other systems^{26,28,29}. It has been suggested that propranolol interaction with phospholipid constituents of the membrane provokes displacement of loosely bound membrane calcium with concomitant changes in calcium pools within the cells²⁵. It is possible, however, that propranolol

Table 2 Meiosis reinitiation by nonsteroidal drugs

Drugs	Concentration range (mM)	Meiosis reinitiation	Interaction with cellular phospholipid constituents*	Ca-ATPase inhibition*
Propranolol-like drugs				
(+) propranolol	0.5-1	+	+25,26,28,29	+30,31,32,66
(-) propranolol	0.5-1	+	+25,26,28,29	+30,31,32
Alprenolol	0.1-0.5	+	+26	
Atenolol	0.1-1	-		
Dichloroisoproterenol	1.0	+	+26	
Metoprolol	0.5-1	+		
Oxprenolol	1.0	+		
Practolol	0.1-1	-	-29	+31,66
Sotalol	0.01-10	-	-28,26	
Local anaesthetics				
Benzocaine	5	-	+67	
Dibucaine	0.5-1	+	+25,67,68	+70
Lidocaine	0.5-1	+	+67,69	+70
Procaine	1-10	-	+67,69	+71
Tetracaine	0.5-1	+	+67,72	+70,73
Drugs acting on CNS				
Allobarbitol	1-5	+		
Amobarbitol	0.5-1	+		
Barbitol	1-5	+		
(+) butaclamol	0.5	+		
(-) butaclamol	0.5	+		
Chlorpromazine	0.5	+	+68,74,75	+70,76
Fenfluramine	1.0	+	+68	
Fluphenazine	0.2	+		
Haloperidol	<5	-		
Imipramine	0.5	+	+68	
Morphine	0.1-5	-		
Naloxone	0.1-5	-		
Nicotine	0.1-10	-		
Phenobarbitol	<5	-	+77	
Phentolamine	0.5-1	+		
Pimozide	0.5-1	+		
Theophylline	<10	-		
Drugs interacting with Ca fluxes				
A 23187	0.005	-	+78	+78
A 23187 plus Mg	0.005	+	+78	+78
Amiloride	<1	-		
Azide (Na)	<1	-		+79
Caffeine	<10	-		
Cromoglycate (Na)	<1	-		
Dantrolene	<1	-		+80,81
Lanthanum	5	+		+70
Methoxyverapamil	0.5-1	+		+70
Mg	<20	-		
Onabain	1	-		
Phenformin	<1	-		
Quinidine	0.5-1	+		+70,74
Ruthenium red	<5	-		+82
Taurine	1	-		
TEA (tetraethylammonium-Cl)	<1	-		
Veratridine	<5	-		
Verapamil	0.5	+		+70

* Data from the literature obtained in various conditions and with different approaches.

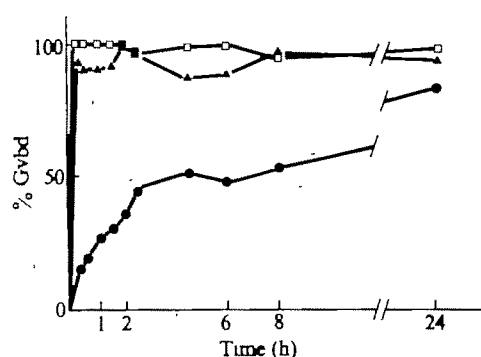


Fig. 6 Effects of progesterone and of polymer-bound progesterone on maturation (% Gvbd) as a function of the duration of hormone exposure. Batches of 100 oocytes from the same female were distributed in test tubes containing 1 ml of incubation medium. At zero time progesterone 0.1 mM ($\square$), progesterone 1 μ M ($\triangle$) or polymer-bound steroid 0.1 mM ($\bullet$) were added. At various incubation times (abscissa), hormones were extensively washed with Barth medium and further incubated. After 24 h Gvbd was scored.

and amphiphilic congeners may influence intracellular Ca^{2+} levels by an action on Ca-ATPase in the oocyte membrane as has been observed in blood red cells³⁰ and in sarcoplasmic reticulum^{31,32}.

Some local anaesthetics induce meiosis, but their activity is not related to their effectiveness as local anaesthetics; thus, benzocaine and procaine are ineffective. It seems likely that the local anaesthetics act by influencing membrane phospholipids, with concomitant Ca^{2+} displacement^{25,33}, as found for propranolol-like drugs. Procaine and benzocaine have a very limited ability to displace calcium in the microsomal system³⁴.

A variety of drugs active on the central nervous system, including neuroleptics (chlorpromazine), antidepressants (imipramine), anorectics (fenfluramine) and some barbiturates, also display maturational activity. Their effect can be attributed to an interaction with membrane phospholipids (and possibly calcium) rather than with stereoselective receptors³⁵⁻³⁷. Phen-tolamine, an α -adrenergic antagonist, is active on oocytes and also shows membrane effects involving lipid metabolism and Ca^{2+} fluxes³⁸. A wide variety of drugs inactive on oocytes (Table 2) do not exhibit membrane phospholipid or Ca^{2+} effects.

Some organo-mercurial compounds³⁹, such as *p*-chloro-mercuribenzoate, have also been shown to induce maturation, possibly through interaction with SH-groups of the membrane, as induction is inversely related to their ability to penetrate the cells.

Calcium and progesterone

The earliest changes following hormone or drug interaction with the oocyte membrane leading to meiosis are unknown. However, there is considerable evidence which suggests that membrane and/or internal calcium distribution is affected.

Among drugs interacting with Ca^{2+} fluxes and/or intracellular exchanges, methoxyverapamil and verapamil were found to be potent inducers of meiosis. Both are tertiary amines and may be regarded as cationic drugs which, as propranolol-type inducers, act by displacing membrane Ca^{2+} (ref. 35), or inhibiting a Ca^{2+} efflux pump in the membrane which maintains low levels of intracellular Ca^{2+} . Inhibition of such a pump would cause the cytoplasmic Ca^{2+} concentration to rise. The positive effect of quinidine, in contrast to those of ouabain and caffeine, is probably attributable to its capacity for displacing calcium from binding sites as in the microsomal fraction of skeletal muscle³⁴. The direct or indirect interaction with Ca^{2+} fluxes of the other drugs listed in the last section of Table 2 are either not selective enough to promote meiosis, or are secondary to other ion permeability changes.

Involvement of Ca^{2+} changes has been also suggested by the action of lanthanum, which induces Gvbd and correlated

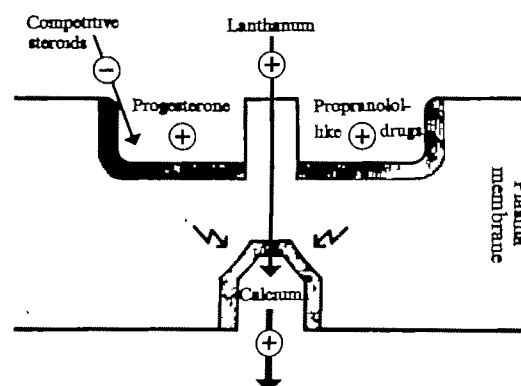
phenomena⁴⁰. La^{3+} has a very high affinity for the Ca^{2+} binding sites of Ca^{2+} -dependent ATPases. Moreover, Gvbd can be provoked by using ionophore A 23187 (if Mg^{2+} or Ca^{2+} is present⁴¹) or specific Ca^{2+} iontophoresis⁴², whereas its induction by progesterone or ionophore is suppressed when intracellular free Ca^{2+} is chelated by EGTA injection⁴³.

Calcium has been proposed as the 'second messenger' of several mitotic factors, and implicated in various changes occurring during cell division. It could then well be involved in progesterone action on oocyte meiotic division. Moreover, calcium is known to inhibit adenylate cyclase activity and its binding to phosphodiesterase activator has been reported. Indeed, early steps of progesterone action may be compatible with either a decrease of cyclic AMP and/or a decrease in the phosphorylation of protein(s) involved in the arrest of meiosis. The former has been observed in *Rana* oocytes (as well, incidentally, as the diminution of cyclic-GMP) (refs 44, 45). (However, the pattern given by our measurements of cyclic AMP in progesterone exposed *Xenopus laevis* oocytes differs somewhat from what has been found in *Rana* (S.S.-S. *et al.*, in preparation).) The latter is suggested by the effect of the injection of purified catalytic subunit of protein kinase (from rabbit skeletal muscle) which inhibits the progesterone-induced maturation⁴⁶; the regulatory subunit or the heat-stable protein inhibitor of cyclic AMP-dependent protein kinase, injected in the absence of progesterone, induces meiotic cell division. We have found⁴⁷ that cholera toxin is a powerful inhibitor of progesterone action. Taken together, these striking results suggest a role(s) for a cyclic nucleotide(s) in the meiotic cell division, possibly dependent on Ca^{2+} changes and leading to an early modification of protein phosphorylation⁴⁸ (clearly distinguishable from the burst of soluble protein phosphorylation occurring later, just before Gvbd⁴⁹).

Three membrane features

The studies reported suggest that three major areas of progesterone action at the surface level of amphibian oocytes merit detailed investigations (Fig. 7). (1) The nature of the interaction site for progesterone and other active steroids seems to be neither a conventional receptor protein nor a 'simple' nonspecific lipidic array. (2) The nature of the calcium binding site and/or ionophoric element, connected in some manner with the putative hormone site, is unknown. (3) The membrane

Fig. 7 The three membrane features implicated in the very early steps of progesterone action are schematically represented, and also seen as pharmacological targets ($\oplus$): agonist, $\ominus$: antagonist). The progesterone site can accept competing antagonist steroids (Table 1). The membrane intrinsic components can be modified by propranolol-like drugs (and other compounds interacting with membrane) at millimolar concentration (Table 2). They activate the postulated calcium site. (The drawing arbitrarily indicates that drugs do interact with membrane elsewhere than in the progesterone site: this is, however, not excluded.) The release of calcium from its membrane site follows steroid agonist interaction in the progesterone site or drug effect on the membrane; it can possibly be provoked by La^{3+} ions



environment is probably of importance for the activity of these hormone and calcium sites. Then perturbation of membrane structure/fluidity will alter their functions. We suggest that the various drugs of the propranolol type modify the oocyte membrane and in turn membrane-bound calcium in the same way as progesterone does.

Conclusions

Xenopus laevis oocytes have long been known to provide a useful tool for investigating several aspects of gene expression⁵⁰. Now, in relation to progesterone-induced meiotic maturation, these cells may contribute to a better understanding of other important problems.

(1) Initiation of meiosis at membrane level. Steroids are active in the resumption of meiosis in all amphibians so far tested and in lower vertebrates such as fish. In starfish, 1-methyladenine is the 'hormone'⁵¹. In mammals⁵², it was shown that one cannot release the oocyte from the follicle without resuming meiosis, and it was hypothesised that either the granular cells produce an inhibitor or that meiosis takes place in association with ovulation, which disrupts cell-cell interaction. The fundamental question is whether the processes leading to meiosis are always initiated at the membrane level and are the same in widely different species, despite differences in the initial trigger.

(2) Mechanisms of hormone actions. The molecular aspects of progesterone-membrane interaction may provide an opportunity to study a new type of target site for steroid hormones in somatic cells. For example, the very early effects of oestrogen on membrane permeability in uterine cells⁵³ may be relevant to such sites. It has already been suggested that steroid hormones act in part at the surface of target cells, and specific interactions have been described^{54,55}. The selective effects of deoxycorticosterone on *Neurospora crassa* permeability to cations⁵⁶ and of progesterone on *Dictyostelium discoideum* development⁵⁷, the specificity of steroid structure in anaesthesia^{58,59} and the physi-

cal effects of steroids on membranes^{60,61} should be further studied. In many systems, steroids are cell division and/or differentiation promoting/regulating factors, but as yet no related mechanistic studies have been carried out. The oocyte-progesterone system may suggest the importance of the interaction between steroids and plasma membrane in this respect, and more work should establish whether some features proposed in 'cell surface modulation'⁶² are involved. This is not to deny that steroid-receptor complexes act in somatic cells at the nuclear locus and that the largest part of the hormonal response is due to this action in the nucleus. The present results with oocytes support the idea that hormone interaction at non-nuclear site(s) may well be complementary to action at the nuclear level in somatic cells and may even be essential for some aspects of hormone activity (cell division). This concept, that a hormone may act at multiple loci in the target cell, has been previously discussed^{63,64}.

Finally, the possible role of calcium ions as second messenger of a steroid signal-membrane interaction is worth testing in other steroid-sensitive systems.

(3) General aspects. In *Xenopus laevis* oocytes, progesterone induces a series of events which are themselves of basic biological importance. For example, the unknown mechanism by which selective protein synthesis is involved at the translational level during progesterone action is of great interest. Mpf, a protein synthesis-dependent product of progesterone action, is remarkable because it promotes all events following meiotic division and it is possible that a similar mitotic promoting factor is to be found in somatic cells undergoing cell division under hormone stimulation.

Xenopus oocytes are arrested cells which may yield much information about events generated at the surface level to biologists who care to expose them to progesterone.

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articles

Slow earthquakes and stress redistribution

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Strainmeters with high sensitivity over long periods have enabled the detection and identification of slow earthquakes: seismic events which produce records similar to those from normal earthquakes except that the time scale for the rupture process is considerably longer. Slow earthquakes provide a mechanism for stress redistribution before normal earthquakes. Stress concentration may take place just hours or days before an earthquake; if it did, this would affect prediction capability.

EARTHQUAKES are generally assumed to result when the gradual stress buildup in a region eventually exceeds some critical local strength. The fracture which develops is thought to propagate at a velocity approaching that of shear waves. Although this is supported by laboratory experiments¹, there have always been observations which cannot be explained by fracture in an otherwise perfectly elastic medium. The time periods between events in a foreshock–aftershock sequence, for example, far exceed the time necessary for elastic redistribution of stress. Clearly, one or more other mechanisms must have a major role in the stress redistribution.

There have been various studies^{2–4} of particular earthquakes inferring coseismic processes with time scales longer than normally associated with earthquakes. However, the evidence for these so-called 'slow' or 'silent' earthquakes has generally been indirect (for example, anomalous long-period spectral behaviour³ or unusually long surface-wave coda⁴) as the time scale of such processes lies outside the passband of conventional seismographs.

A network of Sacks–Evertson borehole strainmeters⁵ was installed along the seismically active Pacific coast of Honshu south of Tokyo which enabled the investigation of the possibility of 'infraseismic' mechanisms for redistribution of stress. These instruments have high sensitivity from zero frequency to several Hz. In addition, tests with nearby explosions (producing accelerations up to g at the instrument) produced no spurious recordings⁵.

New, direct observations of strain redistribution interpreted in terms of events which we call slow earthquakes are reported here. All our observations are consistent with the hypothesis that these slow earthquakes are similar to normal earthquakes in

all respects except for slower rupture velocities and longer rise times. Here we describe slow earthquakes which occur separately from normal earthquakes and which were observed on the recently installed borehole strainmeters or on nearby extensometers. Other kinds of data are also included which indicate that the stress buildup before an earthquake may be non-linear in time. In these cases the concentrations of stress seem to occur in a much shorter time preceding the earthquake than that calculated on the basis of magnitude–precursor–time formulae⁶.

Strainmeter waveforms for normal and slow earthquakes

To understand the characteristics of slow earthquakes, we first examine strain waveforms from normal earthquakes, particularly in the near field (less than 10 fault lengths) where observations of slow earthquakes are most convincing. Figure 1 shows the radial strain field based on a modification of a dislocation model in the form presented by Harkrider⁷. The theory is oversimplified (for example, a point source in an infinite space is assumed) but the results are expected to be qualitatively correct.

The waveforms shown in Fig. 1 can be treated as the superimposition of two parts: a strain step which is proportional to the moment and which falls off as the cube of the epicentral distance, and a 'radiated' pulse which is defined here to include both a term with zero total area which falls off with distance as well as a term whose area is proportional to the moment but which falls off with the square of the distance. Within a few fault lengths (see Fig. 1a) the strain step dominates, while beyond about 20 fault lengths the step would not be detectable—except for an event with an extremely large fault offset.

Figure 2a is a wide-frequency-range borehole strainmeter record from Matsushiro of a nearby small ($m = 4$) earthquake. The compressional wave is followed by a larger shear arrival, and the static offset arrives shortly after S . The basic low-frequency structure is emphasized and the higher-frequency oscillations suppressed in the (effectively) low-pass filtered version of the same record shown in Fig. 2b; this and the record shown in Fig. 3a (a low-pass filtered record of the same earthquake from the Hokushin Observatory near Nagano) show good agreement with the theoretical strain record of Fig. 1c.

Figure 3b shows a strain event recorded by a borehole strainmeter at Irako. Although the record shown is from a

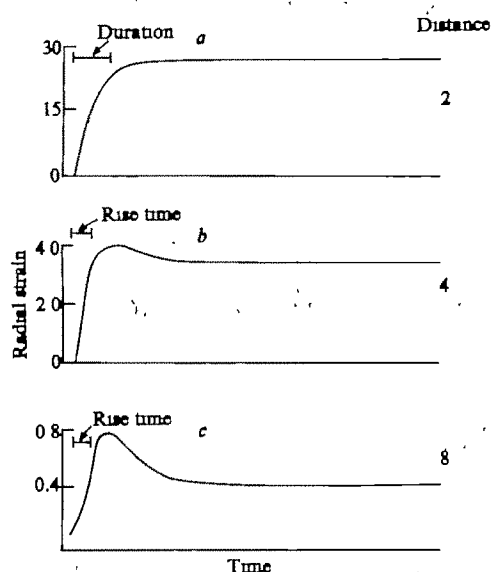
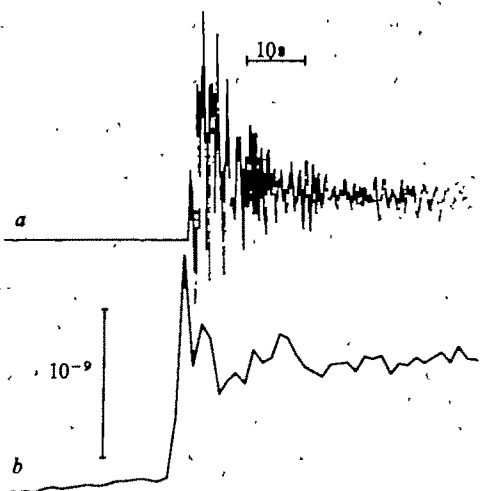


Fig. 1 Theoretical waveforms of radial strain plotted against time of an event at different epicentral distances. *a*, At close distances, there is a relatively slowly rising step. The duration is the time for which movement (rupture) occurs on the fault. *b* and *c*, at greater distances the radiated pulse is observed because it decays with distance less rapidly than does the strain step. The rise time is the time needed for the strain to achieve 86% of its peak value. The time scale is the same in all cases, and the amplitude scales with increasing distance as indicated.

wide-frequency-range channel such as that used for Fig. 2*a*, only low frequencies appear in the arrival. The waveform is similar to that of the earthquake in Fig. 3*a* and to the theoretical waveform in Fig. 1*c*. Many such events have been observed on the recently installed borehole strainmeters as well as on some nearby extensometers. The radiated pulses and strain steps from such events seem to be similar to those from normal earthquakes except that the rise time and duration are about 100 times longer than those for earthquakes of similar moment. It is therefore possible to produce slow-earthquake waveforms which are in reasonable agreement with observation by using normal-earthquake theory with long rise times and low rupture velocities.

Fig. 2 Record from the borehole strainmeter at MAT of an $m = 4.0$ earthquake on 19 September 1973 at a distance of 15 km. *a*, Broadband-response record. *b*, Low-pass filtered record from a different sensor in the same instrument. This sensor has a higher noise level. The magnification of the trace in (*a*) is about one-half that of the trace in (*b*). The traces are slightly offset in time for ease in viewing.



At smaller distances, the theory then predicts records such as shown in Fig. 1*a*. Figure 3*c* shows a slow earthquake with similar waveform. It should be kept in mind, however, that for observations on only a single instrument there is an unresolvable ambiguity between an event being near, slow and of a certain moment or further, slower and of a larger moment.

Events such as those in Fig. 3*b, c* differ from creep events (observed close to a long fault) in that in such events there is no permanent change in the strain field⁸.

The expanded time scale for slow earthquakes means that almost no energy is radiated in the normal seismic frequency band, so that slow earthquakes cannot be detected by seismometer networks used for earthquake location. Even with instruments of high magnification at low frequencies, only nearby events can be clearly identified because theoretical considerations suggest⁹ that the low rupture velocities for these events result in low radiation efficiencies. Hence, such instruments are required in denser networks than now exist to do multi-instrument correlations of individual slow earthquakes.

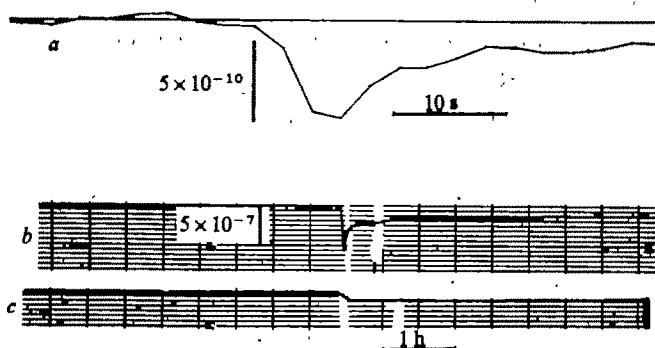


Fig. 3 *a*, Low-pass filtered strainmeter record from the same earthquake as in Fig. 2 recorded at Hokuahin, near Nagano (distance = 15 km, Hokuahin-MAT distance = 13 km). This record can be compared with the theoretical waveform in Fig. 1*c*. *b*, Slow earthquake record for 25 June 1976 from the broadband-response sensor of the borehole strainmeter installed at Irako, on the Pacific coast. Note the similarity between this record and that from a normal earthquake (above) as well as the theoretical waveform in Fig. 1*c*. *c*, Slow earthquake record on 26 July 1976 from Irako. This event is either of similar time scale but at a closer distance compared to the one above or it is slower and at a similar distance.

Foreshocks and aftershocks of slow earthquakes

Shallow earthquakes may have foreshocks and aftershocks which are considerably smaller than the main event. Slow earthquakes may exhibit similar behaviour; Fig. 4 shows a slow earthquake accompanied by a foreshock-aftershock sequence. Relative to the step size, the foreshocks have larger radiated pulses than the aftershocks. If these are all at more-or-less the same distance and have similar rise times, one interpretation is that the foreshocks have higher stress drops. Such a phenomenon has been claimed for some instances for normal earthquake foreshocks and aftershocks¹⁰.

Slow earthquakes as precursors for a normal earthquake

Figure 5 shows the relative locations of the borehole strainmeter at Shizuoka, the Fujigawa extensometers and the epicentre of an $m = 5.5$ earthquake which occurred on 15 June 1976. Also shown are the dates and (relative) amplitudes of strain steps associated with slow earthquakes observed at the two observatories. The Fujigawa extensometers have operated for several years, but slow earthquakes were recorded only during the period January-May 1976. The borehole strainmeter at Shizuoka began operation in early April 1976 and recorded slow

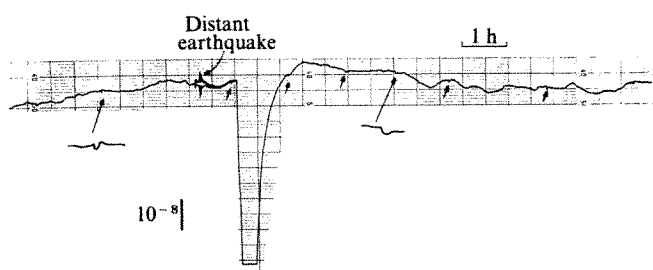


Fig. 4 Irako borehole strainmeter record for 25 August 1976. A slow earthquake, strain = 7×10^{-8} , is shown preceded by foreshocks and followed by aftershocks with strains less than 10^{-9} . Expanded-scale tracings of sample foreshocks and aftershocks are included. The strain record has been high-pass filtered at 25-min period.

earthquakes (of the opposite polarity from those at Fujigawa) from that time until early June. None of the events is recorded at both observatories. Based on the Fujigawa records, the slow earthquakes started about 150 d before the earthquake, which is an often quoted⁶ precursor time for an earthquake of that magnitude.

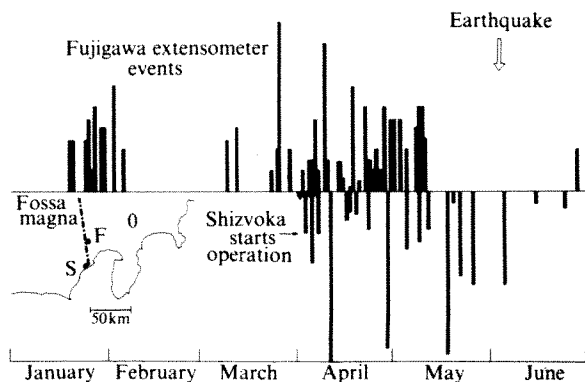
A model consistent with these observations has been suggested by Y. Okada (personal communication). The observatories are on opposite sides and within a few kilometres of the Fossa Magna, a major geological discontinuity (see Fig. 5). Okada notes that the relative amplitudes and polarities of the slow earthquakes at the two observatories could be explained if the slow earthquakes occurred on the Fossa Magna.

Other detections of preseismic stress redistribution

There are other data which indicate that the concentration of stress in an epicentral region may be very nonlinear in time: three examples are given here.

Figure 6 shows the tide-gauge record from Irozaki, on the tip of the Izu peninsula, Honshu, Japan for the day on which a nearby $m = 6.9$ earthquake occurred. In this region, the return time for such an earthquake is many decades. As can be seen in Fig. 6, there is a deviation from the expected tide level apparent about 20 min before the earthquake. This deviation is in the opposite sense to the eventual level change due to the earthquake. An interpretation could be that, due to a nearby slow earthquake shortly before the normal earthquake, there was a buildup of strain of about one-third of that eventually released.

Fig. 5 Time history of the slow earthquakes (recorded on the borehole strainmeter at Shizuoka and on the Fujigawa extensometers) preceding the $m = 5.5$ Yamanashi earthquake. The Shizuoka observatory began operation in April 1976. The amplitude scales for the two instruments are not the same, but the relative polarity of the strain steps is as indicated. Also included is a map showing the relative locations of the two observatories, the epicentre (C) and the Fossa Magna.



Rikitake¹¹ describes a number of tilt changes that have been reported preceding large earthquakes. While his examples establish that large changes of ground level or tilt occurred some days or hours before earthquakes, in most cases there are no instrumental observations which would allow quantitative estimates. One exception is the Tonankai earthquake ($m = 8$) of 7 December 1944.

Levelling surveys in the region of Kakegawa (~150 km north east of the eventual epicentre) showed slight subsidence corresponding to a tilt of 3×10^{-6} rad in the 10 yr preceding the earthquake. On the morning of the earthquake (which occurred at 13 36 LT) a level-survey team was operating north of Kakegawa. Over a 2-h period (up to 12 noon) closure errors of 9 mm over 2 km were found; during this brief period the ground had tilted 4.5×10^{-6} rad in the sense of uplift at Kakegawa. Comparison of a survey immediately after the earthquake with one completed a month before showed a tilt of about 10^{-5} rad in the region north of Kakegawa. This total tilt was in the same

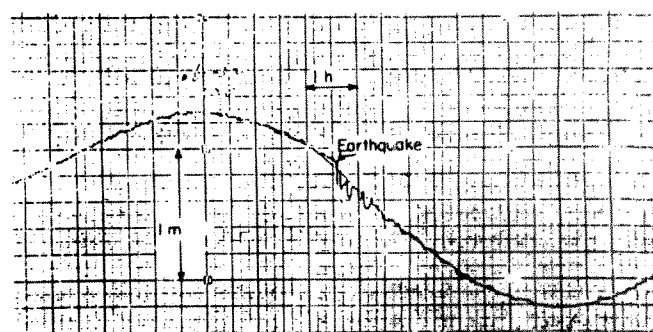


Fig. 6 Tide-gauge record from the tip of the Izu peninsula for 9 May 1974. On this date an $m = 6.9$ earthquake occurred within a few kilometres of the tide gauge. Note that preseismic motion is apparent about 20 min before the earthquake.

sense as the rapid precursory tilt on the morning of the earthquake. Thus this precursory tilt and that due to the earthquake were approximately equal.

A possible interpretation is the precursory tilt was due to a slow earthquake of moment comparable to the normal earthquake.

An $m = 5.2$ earthquake near Hollister, California, on 28 November 1974 was preceded by tectonomagnetic effects¹² and anomalous tilting¹³. Magnetic field changes of about 1.5–2 nT measured at some 16 km from the eventual epicentre occurred over a period of about 2 weeks ending 4 weeks before the earthquake. The time periods involved were much less than the normally found precursory time for an $m = 5.2$ event, and at the distance of the observing stations, there is no anomaly coincident with the quake.

These observations could be explained by slow earthquakes redistributing the regional stress in a manner similar to that suggested by Smith and Johnston¹².

On 14 January 1978, there was an $m = 6.5$ earthquake at 23 km depth near O-Sima Island, Japan. The epicentre was ~33 km from the borehole strainmeter at Irozaki. From the time of its installation in April 1976 through November 1977 the observed strain rate was less than 10^{-7} per month. Six weeks before the earthquake, a compressional excursion of 2×10^{-6} occurred over a period of about 20 d. The strain remained relatively constant at this level until about 4 d before the earthquake, when a dilatational trend began. This provides another example of stress redistribution directly preceding an earthquake.

Conclusions

It is likely that strainmeters and tiltmeters, of adequate stability and sensitivity, installed in active tectonic regions would and

have recorded similar events to the slow earthquakes reported here¹⁴.

The simplest model of events leading to an earthquake is that stress builds up gradually in the eventual earthquake region due to some process such as plate motion. At some stage of stress—and in some areas only—dilatancy may occur. In principle, it is possible to detect this dilatancy through measurements of V_P/V_S , elevation changes and other associated phenomena.

We believe, however, that this is not necessarily the sequence for all large, shallow earthquakes. The available data suggest that while there may be a stress buildup in large regions due to tectonic processes, the stress may not become concentrated in the eventual earthquake region until shortly before the earthquake. One mechanism for this nonlinear in time stress concentration is the slow earthquake.

Two significant implications are: (1) if high stresses may not be concentrated in the source region until possibly a few days or less before even large earthquakes, this would set the effective upper limit to the warning period that a specific prediction could

provide for such events. (2) If there are great slow earthquakes, this would be important in the excitation of the Chandler wobble^{15,16}.

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Aquatic sources and sinks for nitrous oxide

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Oxidation of organic material seems to represent a source for N₂O in a wide variety of aquatic systems. Consumption of the gas, attributed to microbial respiration, is observed for a few specific anoxic environments.

NITROUS OXIDE plays an important part in stratospheric chemistry^{1–4} and may influence the radiative budget of the troposphere⁵. Concern has arisen recently^{6,7} that human activity could lead to a significant increase in the source strength for atmospheric N₂O. Assessment of this possibility is hampered by major gaps in our understanding of processes which regulate N₂O in the natural system.

The gas is thought to originate primarily as a product of microbial metabolism^{8,9}. Stratospheric photolysis can remove at most 10⁷ tons of nitrogen per yr and represents the only currently established atmospheric sink^{9,10}. The magnitude of the global source is uncertain, with particular controversy attached to the role of the ocean.

Hahn¹¹ (also Hahn and Junge¹²) concluded, from limited measurements of dissolved N₂O, that the ocean should represent a major source for atmospheric N₂O, contributing as much as 10 times the quantity of gas consumed in the stratosphere. McElroy *et al.*¹³ argued, from consideration of marine productivity, that the oceanic source should be small and raised the possibility that microbial respiration in conditions of low oxygen could offer an important sink for marine N₂O. They speculated that consumption of N₂O might exceed production on an integrated basis for the world's oceans.

This article summarises recent results which attest to the complexity of the aquatic nitrogen cycle as it affects N₂O. We present data from Central and South East Tropical Regions of the Pacific Ocean, and from Chesapeake Bay. Our results support the view^{11,14,15} that oxidation of ammonium and amino nitrogen, nitrification, should represent a major source for marine N₂O. They allow a quantitative estimate for the relevant source strength, implying a global yield for oceanic N₂O of less than about 10⁷ tons N yr⁻¹. Data from the South-east Tropical Pacific and from Chesapeake Bay demonstrate consumption of dissolved N₂O in conditions of low oxygen, as observed by others^{15,16}. There is no evidence to date, however, for consumption of atmospheric N₂O by the open ocean. We report preli-

minary observations indicating consumption of atmospheric N₂O by a number of aquatic systems, including a freshwater pond and a tidal saltmarsh.

Central Pacific

The data discussed here were collected between 2 and 18 December 1977 on the Norpax Leg II cruise of the RV Kana Keoki. Samples were taken to depths of 1,000 m every degree of latitude along the 150° W meridian, between 17° S and 20° N, and were analysed for temperature, salinity, O₂, N₂O, NO₂⁻, NO₃⁻, NH₄⁺, PO₄³⁻ and SiO₂. Analytical techniques are discussed elsewhere^{17,18}.

Cross sectional contours for N₂O, PO₄³⁻ and NO₃⁻ exhibit several similar structures (Fig. 1). Highest concentrations of N₂O are observed at 9° N at a depth of 129 m in an intense feature associated most probably with upwelling in the north tropical divergence zone¹⁹. This feature may be seen also in the contours for PO₄³⁻ and NO₃⁻. Highs in N₂O at 17° N (128 m) and 4° S (502 m) are similarly correlated with structure in PO₄³⁻ and NO₃⁻.

Concentrations of N₂O in surface waters are generally close to equilibrium with the atmosphere. Largest surface concentrations are observed in the zone of high productivity associated with the equatorial upwelling, where N₂O is supersaturated with respect to air by a factor of ~1.4.

On average, surface waters are supersaturated with respect to the atmosphere by a factor of ~1.25 (±0.1), equivalent to an excess of N₂O equal to 38 ± 10 ng N l⁻¹. The uncertainty in this quantity reflects imprecision in the available solubility data, errors associated with the application of these data to seawater, and inevitable uncertainties introduced by subtraction of nearly equal experimental quantities.

Highest concentrations of N₂O in Fig. 1 correspond to regions of low oxygen. It is of interest to consider the relationship between ΔN₂O and ΔO₂, where ΔX defines the excess of X with respect to the atmosphere at the local water temperature and salinity. The quantity ΔN₂O provides a measure of the net accumulation of N₂O in a water mass since its last contact with the air. By the same token -ΔO₂ provides a cumulative record of the net quantity of oxygen consumed over the same period due to oxidation of organic material.

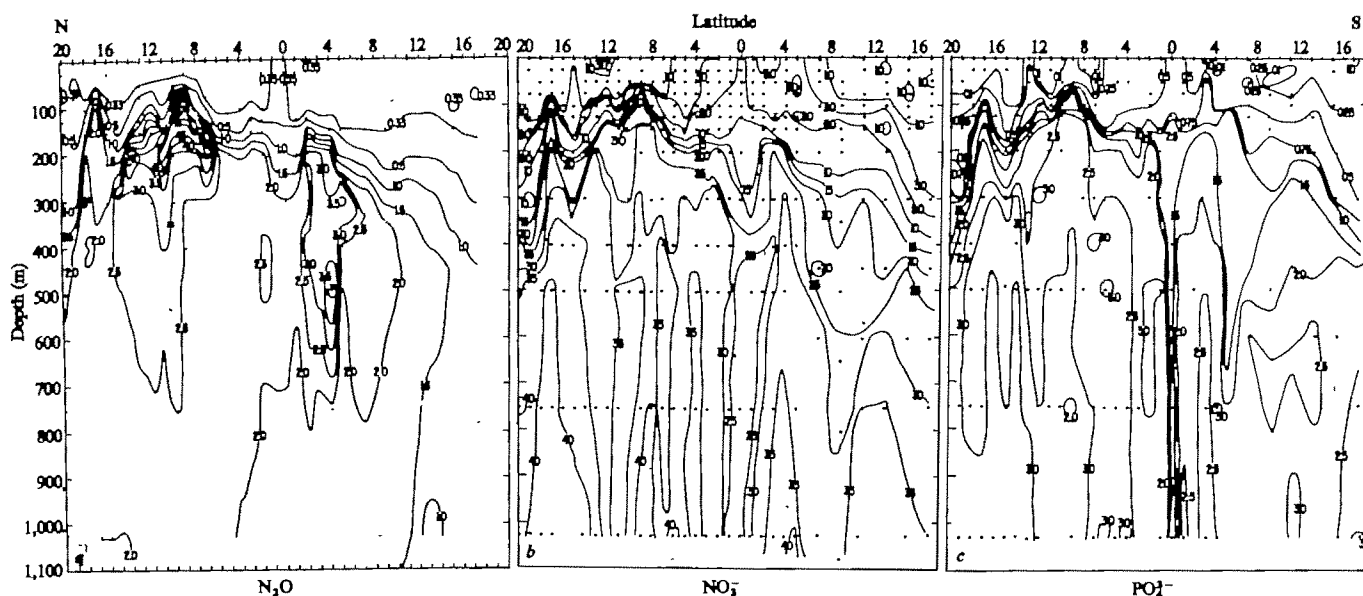
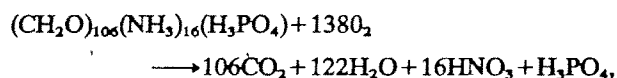


Fig. 1 Cross sections for *a*, N_2O ($\mu\text{g l}^{-1}$); *b*, NO_3^- and *c*, PO_4^{3-} ($\mu\text{g-atm l}^{-1}$) along the 150°W meridian in the tropical Central Pacific Ocean. Experimental precision is better than 3% for N_2O and $0.1 \mu\text{g-atm l}^{-1}$ for NO_3^- and PO_4^{3-} .

Figure 2a shows a plot of $\Delta\text{N}_2\text{O}$ against $-\Delta\text{O}_2$ (the apparent oxygen utilisation or AOU). We include here all of the data in Fig. 1, except for results from active regions at 17°N and $8-13^\circ\text{N}$ (above 200 m) and from 2 to 4°S (above 500 m). The results indicate (see also ref. 14) that a linear relationship may exist between $\Delta\text{N}_2\text{O}$ and $-\Delta\text{O}_2$, and that this relationship may hold over extensive regions of the world's oceans. Indeed data from active zones may also exhibit linear dependence of $\Delta\text{N}_2\text{O}$ on $-\Delta\text{O}_2$ (Fig. 2b). The value for $\Delta\text{N}_2\text{O}$ is, however, larger in this case for any particular choice of $-\Delta\text{O}_2$.

The linear behaviour exemplified by Fig. 2 suggests that production of N_2O , at least for portions of the ocean below the mixed layer, may occur primarily as a byproduct of the oxidation of organic material¹⁴. Production of N_2O during nitrification, observed in culture for both soil²⁰ (*Nitrosomonas europaea*) and marine (*Nitrosococcus oceanus*) organisms (unpublished data from this laboratory) offers a plausible explanation. If we assume that the C:N:P ratios in marine organic material are similar to values quoted by Redfield *et al.*²¹, 106:16:1, and assume further that the stoichiometry of decay may be represented by



then the data in Fig. 2 may be used to estimate the corresponding yield for N_2O . The behaviour exhibited in Fig. 2a suggests that 1 molecule of N_2O may be formed on average for every 4,700 carbon atoms which undergo oxidation.

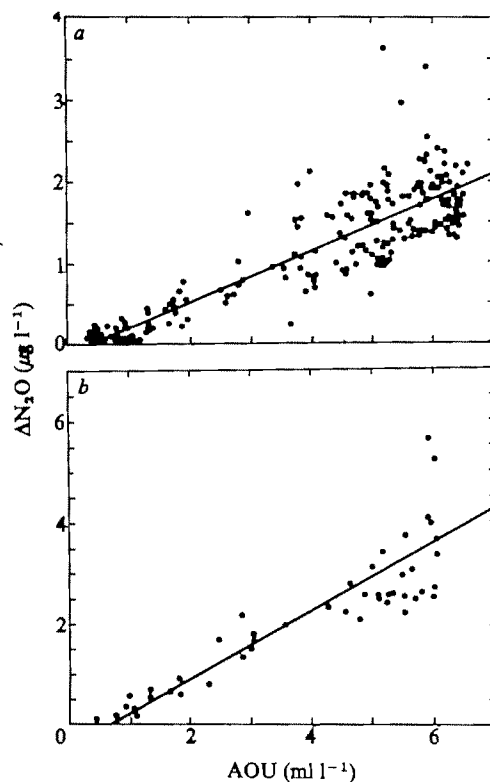
The difference in slopes exhibited by the data in Fig. 2a and b might be attributed to various factors—differences in the C:N:P ratios of organic material; intrinsic differences in the yield for production of N_2O ; *in situ* production and loss of O_2 due to photosynthesis and respiration; complications due to admixture of different water masses; and additional sources of N_2O in active regions associated either with denitrification or with assimilatory reduction of nitrate. It seems that $\Delta\text{N}_2\text{O}$ may be an increasing function of temperature at given AOU. The relation

$$\Delta\text{N}_2\text{O} = -0.46 + \{0.180 + 0.027T\} \text{ AOU}, 25 > T > 5^\circ\text{C}$$

with $\Delta\text{N}_2\text{O}$ and AOU measured in units of $\mu\text{g l}^{-1}$ and ml l^{-1} respectively and with T in $^\circ\text{C}$, provides an excellent fit to all of the data except results from the active region between 8 and 11°N . The root mean square deviation of the data from values

predicted on the basis of the above relation is only $0.3 \mu\text{g l}^{-1}$ for 238 observations characterised by an average value of $\Delta\text{N}_2\text{O}$ equal to $1.4 \mu\text{g l}^{-1}$. The higher yield for production of N_2O at elevated temperature is consistent with temperature sensitivities observed in the laboratory by Yoshida and Alexander²⁰.

Fig. 2 N_2O observations (Fig. 1) expressed as excess N_2O plotted against apparent oxygen utilisation. Most of the data are shown in (a) but data from the active regions at 17° , $13-8^\circ\text{N}$, and $2-4^\circ\text{S}$ are plotted separately in (b). The straight lines are least-squares fits, which yield r.m.s. deviations of 0.37 and $0.6 \mu\text{g l}^{-1}$ for (a) and (b) respectively. Data points from the mixed layer ($\text{AOU} < 0.3 \text{ ml l}^{-1}$) are not included. The measured concentration of atmospheric N_2O was 291 parts per 10^9 (by volume) (p.p.b.).



Peruvian upwelling

An extensive series of measurements was carried out off the coast of Peru from the RV Knorr between 20 and 31 March 1978. A representative depth profile, including data for temperature, N_2O , O_2 , NO_2^- , NO_3^- and NH_4^+ , is given in Fig. 3. For comparison, concentrations of N_2O expected on the basis of the temperature dependent formula quoted above are shown.

The empirical relation provides excellent agreement with N_2O measurements below 300 m and is in accord also with observations at 30 and 50 m. A major discrepancy exists between 100 and 300 m. Comparison of observed and calculated profiles suggests that consumption of N_2O may be important in this zone. Indeed the observed concentration of N_2O is undersaturated with respect to the atmosphere between 150 and 250 m, a region in which oxygen concentrations lie below 0.1 ml l^{-1} .

The zone of low O_2 and N_2O includes a maximum in the concentration of NO_2^- , a feature attributed by others^{15,22} to reduction of NO_3^- . The minimum in N_2O almost certainly reflects respiration of the gas at low concentrations of O_2 as suggested by McElroy *et al.*¹³ and by Cohen¹⁶. It is consistent with a model²² which invokes a major role for denitrifying bacteria at concentrations of oxygen below 0.1 ml l^{-1} .

Further support for the role of denitrifying bacteria may be inferred from incubation experiments carried out as part of the present sequence of observations. Most samples for N_2O analysis were killed immediately with $HgCl_2$. A number of samples, however, were incubated live, in 60 ml BOD bottles. Some of these samples were treated with trace concentrations of C_2H_2 , a procedure known²³ to inhibit reduction of N_2O by denitrifiers. Samples of water from regions of low O_2 , treated with C_2H_2 , showed generally high concentrations of N_2O following 18-day incubation intervals. In one case a sample, with an initial concentration of N_2O equal to $1.3\text{ }\mu\text{g l}^{-1}$, developed a concentration of N_2O of $4.3\text{ }\mu\text{g l}^{-1}$ within an hour of application of C_2H_2 . The concentration of N_2O had reached $191\text{ }\mu\text{g l}^{-1}$ after 18 days, indicating accumulation of N as N_2O in an amount equivalent to ~30% of the starting concentration of NO_3^- . Results from eight other C_2H_2 treated incubation experiments showed 18-day N_2O concentrations in the range 3.3 to $47.0\text{ }\mu\text{g l}^{-1}$. Net consumption of N_2O was exhibited by all samples incubated without C_2H_2 .

Other systems

Figure 4 summarises N_2O observations taken in Chesapeake Bay during July 1977. The data were acquired in waters with total depths in excess of 13 m, and show clear evidence for consumption of N_2O below the seasonal thermocline.

There appears to be an abrupt change in the behaviour of N_2O when the concentration of O_2 falls below 1.4 ml l^{-1} . Waters are then undersaturated in N_2O with respect to air. Indeed at several stations, concentrations of both O_2 and N_2O were observed to lie below present detection limits of 0.1 ml l^{-1} and $0.02\text{ }\mu\text{g l}^{-1}$ respectively.

Fig. 3 Depth profiles for N_2O , mineral nitrogen, oxygen and temperature for a station at $15^{\circ}10.6'S$, $75^{\circ}35.5'W$, in the region of Peruvian upwelling. The dashed line represents the relationship $\Delta N_2O = -0.46 + 0.180 + 0.027 T\text{ AOU}$.

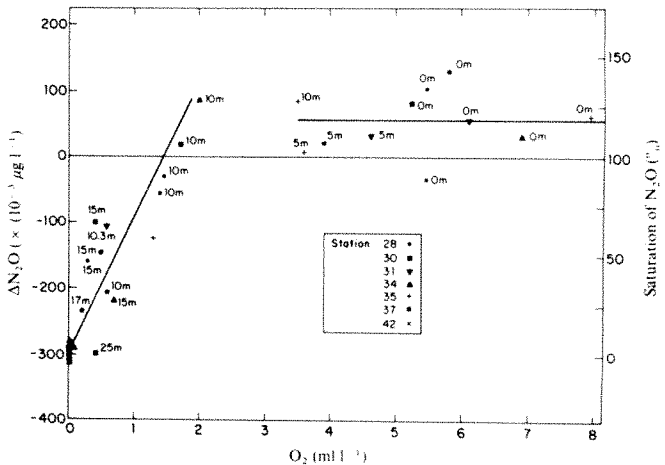
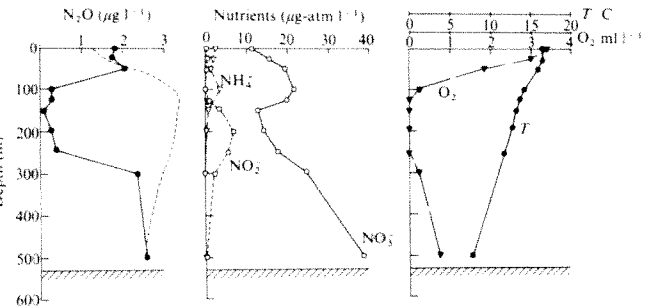
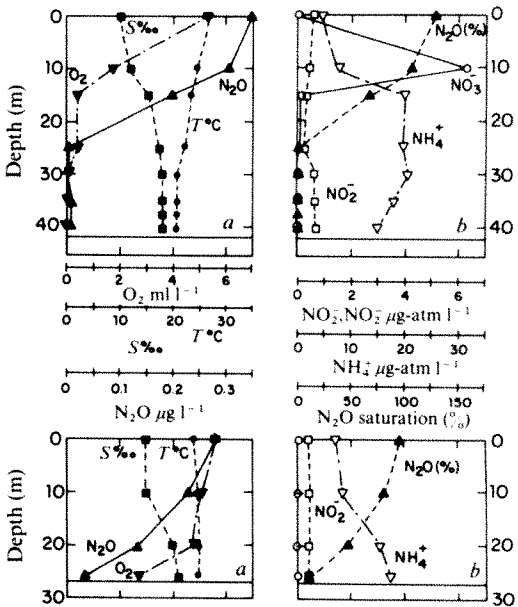


Fig. 4 N_2O and O_2 data for Chesapeake Bay. The % saturation of N_2O is given relative to the observed atmospheric concentration of 291 p.p.b., for $T \approx 27^{\circ}C$.

Depth profiles for N_2O , O_2 , temperature, salinity and mineral nitrogen are shown for two stations in Fig. 5. Station A, included in Fig. 4, illustrates the rapid decrease with depth below the thermocline observed for both N_2O and O_2 . Note that NH_4^+ is the dominant form of mineral nitrogen at all depths in these waters. Station B, occupied following a period of cool windy weather, shows evidence for transient breakdown of the thermocline, with associated mixing of deep and shallow waters. Note in this case that surface waters seem to be undersaturated in N_2O with respect to the atmosphere, suggesting that in summer the Chesapeake may represent at least a transient sink for atmospheric N_2O following periods of rapid vertical mixing. A more extensive sink may occur in anoxic muds associated with tidal saltmarshes as indicated by preliminary studies of a marsh on the margin of the bay at the Choptank River. Waters draining this marsh at low tide were undersaturated in N_2O by 30% with respect to air.

Fig. 5 Depth profiles for N_2O , O_2 , T and S in Chesapeake Bay for station (a), occupied 14 July 1977, and nearby station (b), occupied 27 July 1977. Weather was generally warm and stable, except for a cool windy period 25–26 July. Note undersaturation of N_2O in surface water and nearly isothermal structure at station (b). Location of (a) is $38^{\circ}50'05''N$; $76^{\circ}23'53''W$; location (b), $38^{\circ}40'15''N$; $76^{\circ}25'00''W$.



Consumption of N_2O seems to arise for at least one other system at oxygen concentrations below 1.4 ml l^{-1} . Figure 6a shows a summary of results obtained in a diurnal study of a shallow pond (denoted pond A) in Concord, Massachusetts, a system reported elsewhere¹⁷ to represent a sink for atmospheric N_2O . The present study confirmed that this pond was undersaturated in N_2O over a complete diurnal cycle. Concentrations of O_2 are relatively high during the day, reflecting a strong photosynthetic source. Concentrations of N_2O increase with time when O_2 concentrations are above 1.4 ml l^{-1} , reflecting, presumably, infusion of gas from the air. Figure 6 gives clear evidence for consumption of N_2O at oxygen levels below 1.4 ml l^{-1} . In contrast to pond A, the adjacent pond B remains well oxygenated through the night. It functions as a source of atmospheric N_2O and exhibits a relationship between N_2O and O_2 similar to that observed in the Central Pacific (see Fig. 6b).

It is probable that the sinks observed for N_2O in both the Chesapeake bottom water and in the pond discussed above, reflect utilisation of the gas as an electron acceptor by micro-organisms functioning at low levels of oxygen. Alternate elec-

tron acceptors, such as NO_3^- and NO_2^- , are present in relatively low concentration in these systems, in contrast to the Peruvian upwelling regions where NO_3^- and NO_2^- are relatively abundant. It would be tempting to attribute consumption of N_2O in all these systems to denitrifying bacteria. The hypothesis that denitrifying bacteria may effect removal of N_2O is supported for Peru by the inhibition experiments described earlier. Further work is necessary to clarify the nature of the sinks operative in the pond and bay.

Conclusions

Data from the Central Pacific, Peru, Chesapeake Bay and various ponds and rivers demonstrate that aquatic systems contain both sources and sinks for N_2O . Nitrification may represent a dominant source for N_2O in both freshwater and saltwater systems. Observations from the Pacific, elaborated above, and from various rivers and ponds^{17,18} (see also Fig. 6b) support this conclusion. Present observations suggest that approximately 1 mol of N_2O is formed for every 700 mol of NH_4^+ which undergo oxidation.

A similar yield was observed in the laboratory for soils at comparable pH by Bremner and Blackmer²⁴, suggesting that this yield may have wide application. Indeed nitrification could represent a dominant global source for atmospheric N_2O .

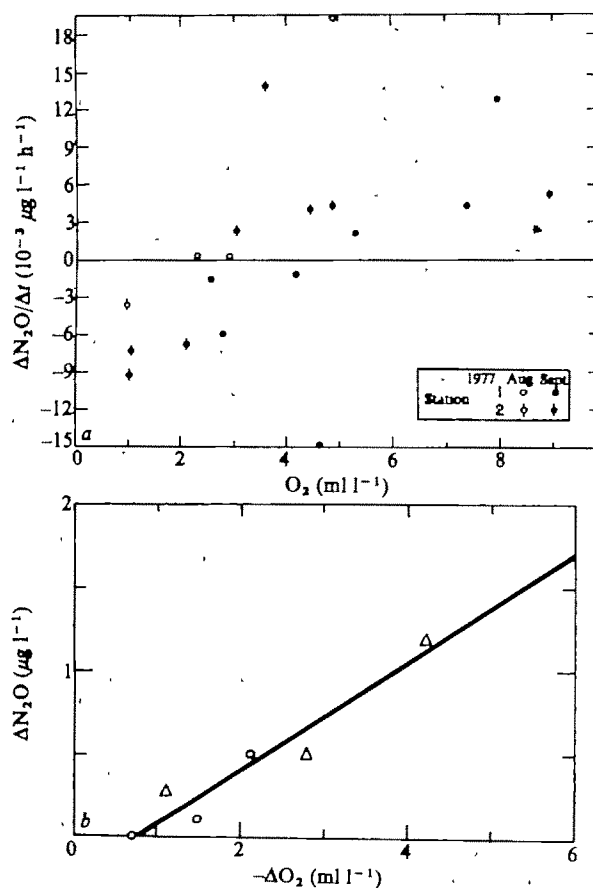
The marine contribution to atmospheric N_2O should not exceed $10^7 \text{ tons N yr}^{-1}$. This follows from the assumption of a C:N ratio equal to 6.6 for the marine biosphere²¹. It applies if the rate for marine fixation of carbon^{25,26} is $2 \times 10^{10} \text{ tons C yr}^{-1}$ and if one can ignore assimilation of reduced forms of N by phytoplankton. A source of $\sim 10^7 \text{ tons N yr}^{-1}$ is consistent with most of the available marine data and is in accord with the Pacific surface measurements discussed here.

As discussed by McElroy *et al.*¹³, it is difficult to derive a reliable value for the global source strength of N_2O based on limited data from surface ocean waters. However, applying the conventional procedure^{11,12} to our data for the surface waters of the Central Pacific gives an estimate of a marine source for atmospheric N_2O of $7 \times 10^6 \text{ tons N yr}^{-1}$ between latitudes $\pm 20^\circ$. To make this estimate one must assume negligible time variability in the concentration of dissolved N_2O in surface water, zonal uniformity in the excess of its concentration with respect to the atmosphere, and a constant piston velocity. From our own data (Figs 1 and 3) it seems unlikely that these assumptions apply to the world oceans, although the resulting estimate is coincidentally close to the upper limit provided by the oxidation model. The multiple equilibration procedure used here provides from each analysis a measure for the solubility of N_2O in seawater. Our data suggest a solubility perhaps 10% larger at 25°C than that deduced from ref. 27 and would imply therefore that the source estimated from the thin-film model should be reduced by 30%. Note that the final concentrations of dissolved N_2O deduced from our analyses are independent of the solubility coefficient for the gas.

Microbial respiration in conditions of low oxygen can represent a sink for atmospheric N_2O . The importance of the process on a global scale is not clear. It has been demonstrated here only for relatively small scale environments. Results from the Chesapeake marsh are intriguing, however, in that coastal marshes and wetlands occupy significant areas of the globe, about $2 \times 10^6 \text{ km}^2$, comparable to areas occupied by regions of intensive coastal upwelling. Further studies are obviously required to quantify the part which biological uptake may play in the global budget of N_2O .

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Fig. 6 a, Time rate of change for the concentration of N_2O in Concord pond A, shown as a function of dissolved oxygen. The pond is about 0.4 m deep and is stagnant, with no surface outlet. The water contained abundant flora but had negligible mineral nitrogen and was undersaturated in oxygen and nitrous oxide throughout the experimental period. b, Variations of N_2O and O_2 for Concord pond B. Quantities plotted represent differences between concentrations measured during the night, and concentrations measured at sunset, a procedure adopted to avoid confusion due to photosynthetic production of oxygen. Pond B contained appreciable dissolved NO_3^- ($\sim 10 \mu\text{g-atm l}^{-1}$). It is located adjacent to pond A and has a surface inlet and a surface outlet, with a water retention time of ~ 3 days. N_2O was supersaturated with respect to the atmosphere at all times, while concentrations of O_2 ranged between 3 and 7 ml l^{-1} . The best-fit linear relation between N_2O and O_2 is given by $\Delta N_2O = -0.235 (\pm 0.16) - \Delta O_2 \times 0.32 (\pm 0.06)$, close to the average line for all data from the Central Pacific.



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Nautiloid growth rhythms and dynamical evolution of the Earth–Moon system

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Daily growth lines and lunar monthly septa are formed in Nautilus pompilius Linnaeus shells. The number of days per lunar month determined using fossil shells has increased dramatically during the last 420 Myr, indicating that during this period the Moon revolved more rapidly and was much closer to the Earth than has previously been expected.

THE hypothesis that the Moon and Earth have been closer in Phanerozoic times has received considerable support from studies of the growth cycles of corals^{1,2}, bivalves^{3–8}, brachiopods⁹ and stromatolites^{10,11}, as well as from astronomical studies of tidal friction^{12–14}. These studies yield information about the rotation rate of the Earth, or the number of days per year, which is consistent with a closer and faster revolving Moon in earlier times. Tidal friction, by which bulges raised in the oceans by the Moon exert a torque on the Moon, implies the gradual expansion of the lunar orbit as well as an increase in the length of day with time. Both of these predictions have been confirmed for recent years by occultation measurements¹⁵, and for historical times by studies of ancient eclipses¹⁶. Unfortunately, the past dynamics of the Earth–Moon system cannot be derived solely from theoretical calculations based on recent and historical astronomical observations since tidal friction is critically dependent on sea level and continental configurations, both of which have changed markedly in the geologic past. Since an accurate charting of lunar orbital evolution has profound implications on lunar origin theories and on cosmological theories¹⁷ which predict lunar orbital expansion as a consequence of a varying gravitational constant, another approach is needed.

One viable approach to prehistoric lunar dynamics is through palaeobiology¹⁸. Nautiloids are organisms which possess a long evolutionary history (~500 Myr), but until now, they have not been investigated as possible geochronometers. We shall examine the hypothesis that there are daily and lunar monthly growth records preserved in the shells of *Nautilus*, and also in the fossil nautiloid shells beginning in the Ordovician, and discuss the implications for the evolution of the Earth–Moon

system. This synthesis suggests that the rate of lunar orbital evolution has been much greater in the past 420 Myr than short-term astronomical studies and theoretical calculations indicate.

Growth of *Nautilus*

Although nautiloid fossils are found throughout the world, the chambered *Nautilus* survives in the southwestern Pacific Ocean as the only genus of externally shelled cephalopods. Observations of *Nautilus in situ* are limited because of its nocturnal habits and its habitat—depths of up to 400 m and away from land masses. Observations on juvenile *Nautilus* are extremely limited²⁰. Thus, our study of *Nautilus* growth relies primarily on a study of shell macro and micro-structure, rather than on observations of the living *Nautilus* in its natural surroundings.

As *Nautilus* grows, convolutedly coiling its shell in the shape of a logarithmic spiral (Fig. 1), it incorporates two kinds of rhythmically repeating structures. One is the secretion of the outer shell at the aperture: miniscule incrementations of calcium carbonate occur in sets of three to five, separated from preceding and succeeding sets by a ridge known as a growth line. The other rhythmic pattern is septation, by which the parts of the shell abandoned by the animal are partitioned off into chambers.

The aperture receives new growth increments at the margin, approximately half a revolution ahead of the chamber wall. The organism periodically pulls itself towards the extended aperture and deposits another septum behind it. No direct time association, therefore, exists between these two growth measures. A span of months separates the deposition of a chamber wall and the growth lines counted for that chamber. However, throughout the animal's life these septa are placed at remarkably regular intervals in the shell (Fig. 1).

As shown in Fig. 2, the number of growth lines per chamber length in *N. pompilius* is 30 ± 2 , a relationship that seems to hold throughout ontogeny. The present length of the lunar synodic month is 29.53 days. This suggests that one growth line is deposited each day and one septum is developed each lunar synodic month. While the growth rates and life habits of *Nautilus* remain to be studied extensively, the following lines of evidence suggest that this timing is reasonable. (1) Other molluscs show tidally induced or circadian skeletal banding at about the same rate of growth^{3–6}. (2) The activity cycle and

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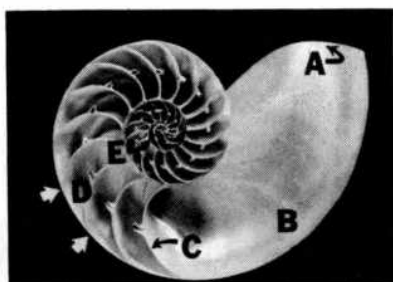


Fig. 1 Longitudinally sectioned shell of *Nautilus pompilius* showing **A**, margin of aperture where growth lines are secreted; **B**, body chamber where animal lives; **C**, septum or chamber wall; **D**, chamber space. The number of growth lines per chamber length (between arrows) can be counted using a scanning electron microscope. These counts reveal a constant number of growth lines per chamber. If our hypothesis is correct, the chambers are secreted at lunar monthly intervals.

depth migration²¹ (movement into shallow waters at night) of *Nautilus* and the attendant stress on physiology makes the expression of a daily rhythm in the skeleton seem very likely, particularly when considering the effect of periodic respiratory changes upon growth line formation²². (3) Experimentally reared cephalopods (*Sepia* and *Sepiella*) showed daily lines²³. And (4), that successive septa are formed at intervals of at least several weeks has been supported by studies of the gas content of *Nautilus* chambers^{24,25}.

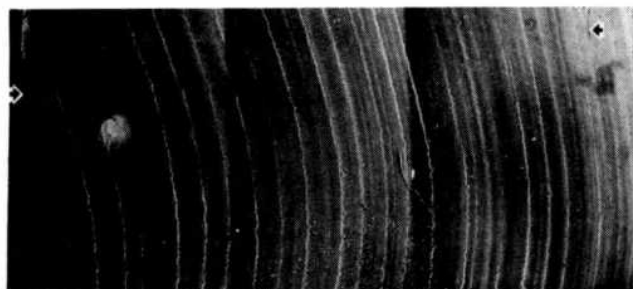
Therefore, we accept as a working hypothesis the idea that the rate of secretion of growth lines is circadian, and chambers circa-lunar. Thus, one rhythm is tied to the Earth-Sun relationship, the other to the Earth-Moon.

Growth line counts

The chambered cephalopods, including nautiloids, are probably among the most accurately dated of all fossils, due to their rapid evolution and extensive preservation in sedimentary deposits around the world. Fossil nautiloid growth lines are quite easily discerned, well preserved, and unambiguous, as shown in the photographs of growth lines (Figs 4, 5). The growth rhythm of nautiloids appears to manifest itself not just in regional settings. For example *Aturia* sp. found in Australia exhibits essentially the same growth rhythm as *Aturia curvilineata* found in Florida (Table 1). As nautiloid growth lines are believed to be daily in nature and not tide-induced, determination of the nature of the tidal cycles present in the animal's habitat is unnecessary. All of the above factors fulfill criteria essential for reliable and useful nautiloid growth line counts in geochronometry.

Growth lines are present on many superbly preserved fossil specimens. Growth lines on *N. pompilius* were examined and photographed using a scanning electron microscope or a stereoscopic optical microscope with oblique light to accentuate the growth lines.

Fig. 2 Scanning electron micrograph of the growth lines present on the outer shell of a recent *Nautilus pompilius*, ventral section. The number of growth lines per chamber (between arrows) averages 30. The number of days per lunar synodic month is 29.53.



The number of growth lines per chamber was counted on 184 different chambers of 9 recent and 29 fossil nautiloid specimens (Table 1). The specimens represent nine geologic periods and a range of about 420 Myr. Figure 1 illustrates the structure of the *N. pompilius* shell, showing the location of growth line counts. Figure 2 reveals the distinct growth lines on the ventral side. All of the counts of growth lines per chamber for consecutive undamaged chambers of recent *N. pompilius* approximated 30 (Table 1). For individual chambers, the number of growth lines ranged from 28 to 34; the average for all chambers of all specimens was 30 ± 2 . The radial lirae, or ridges, on the internal layer of the initial whorl of three *N. pompilius* specimens were counted, consistently revealing 26–32 lirae per chamber (Fig. 3), an average of 29 per chamber per specimen.

As we go back in geologic time, the number of growth lines per chamber decreases. The number of growth lines per chamber had increased from 9 at 420 Myr ago to 30 today (Table 1). Three broad conclusions can be drawn from our data. First, the number of growth lines per chamber is constant for the various chambers in a single specimen. Second, many of the nautiloids living at any given time secrete the same number of growth lines per chamber. This seems to imply a ubiquitous rate of septal and outer shell deposition at any particular geologic time. Third, and most important, the number of growth lines per chamber decreases with increasing geologic age (Fig. 6).

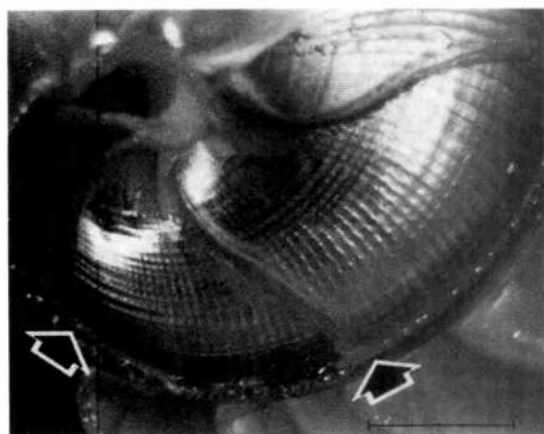


Fig. 3 Ventral section of *Nautilus pompilius* exposing the radial lirae on the interior of the chambers of the initial whorl. The lirae correspond to external growth lines, and their number/chamber averages between 28 and 30. Arrows mark chamber walls. Scale bar, 0.5 cm.

Application to Earth-Moon system

By assuming that septal secretion was controlled in the same way for fossil nautiloids (on a lunar synodic monthly basis), we can calculate the period of the Moon's orbital motion and the Earth-Moon distance up to 420 ± 10 Myr in the past, the age of our earliest specimens. The calculations made involve the following assumptions: (1) The length of the sidereal year is assumed to be constant by virtue of the large moment of inertia of the Earth around the Sun²⁶. (2) The Earth's period of rotation is assumed to have increased at the rate of 25 s per million years, the consensus result indicated from ancient eclipse studies¹⁶, some astronomical studies²⁷, and from palaeontological studies of fossil corals¹. (3) The mass of the Earth and Moon have remained constant for the past 500 Myr. We assume also that the gravitational constant is unvarying. (4) To simplify calculations, the barycentre of the Earth-Moon system is taken as the centre of the Earth.

Although the number of days in the synodic month seems to be recorded by nautiloids, for our orbital calculations the

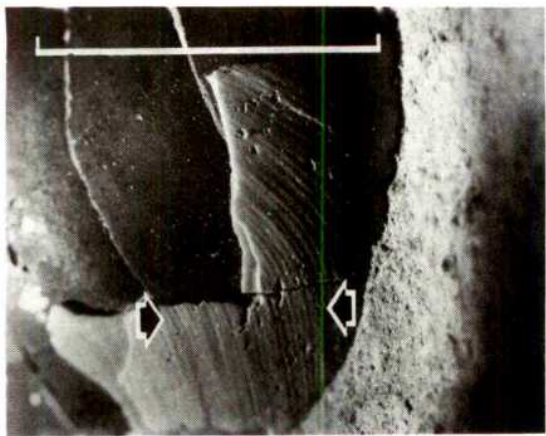


Fig. 4 Close-up of the outermost shell layer with growth lines on a remarkably preserved *Eutrophoceras dekayi* from the Pierre Shale of South Dakota (Upper Cretaceous, ~69.5 Myr ago). The number of growth lines per chamber (between arrows) is 22 ± 2 . If chamber secretion involves a lunar monthly rhythm, then the Moon circled the Earth every 22 days, 69.5 Myr ago. Scale bar, 1 cm.

sidereal month must be used. Following Runcorn²⁸, the relationship between the synodic month S_n , the sidereal month S , and the present year length Y , can be expressed for any geologic time as:

$$S = S_n / (1 + S_n / Y)$$

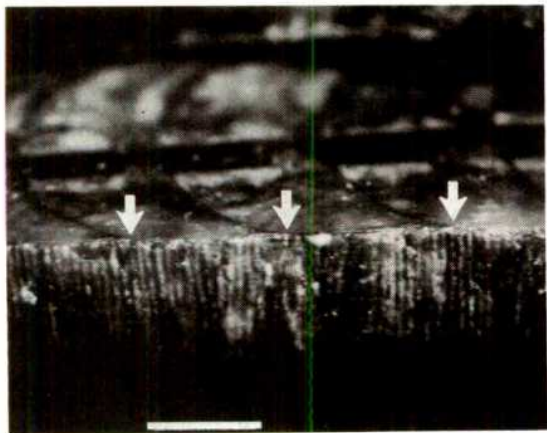
To avoid confusion originating from changes in the day length, these values are expressed in hours. Applying Kepler's third law, neglecting eccentricity, yields the semi-major axis of the Moon's orbit for any geologic time.

Mode of life of *Nautilus* and nautiloids

Nautilus pompilius Linnaeus does not crawl on the ocean bottom, but instead is an active epibenthic swimmer. Several lines of evidence point to a similar mode of life for most fossil nautiloids, particularly the development of ribbing and coiling of shells and of chambers with gas and cameral deposits to maintain the body chamber in a horizontal position. This orientation would be essential for any nautiloid to propel itself effectively.

Three species, *Welleroceras liratum* Miller and Furnish, *Pseudorthoceras knoxense* McChesney, and *Orthoceras unicaera*, did not fit the general trend shown in Fig. 6. However, counts for these shells did possess integral multiples of the predicted number of growth lines per chamber length. One

Fig. 5 Dorso-ventral section of an orthocerid ~326 Myr old. The arrows mark the different chamber walls. There are a constant 15 ± 1 growth lines per chamber, the same number possessed by other nautiloid genera from almost time equivalent Mississippian formations. Scale bar, 1 cm.



species, *Pseudorthoceras knoxense*, had growth lines grouped together in threes, and the number of growth line groups was equal to the growth line counts of other specimens of that time. The evidence suggests that the modes of life of these three nautiloid species did not resemble that of modern *Nautilus*. Though depth ranges of fossil cephalopods are difficult to establish²⁹, we suggest, based on morphologic³⁰ and palaeo-ecologic studies³¹, that these three, each displaying weak septa, may have been restricted to a relatively shallow shelf and inland sea habitat (50–100 m depth). In addition, the *Welleroceras liratum* may not have been able to maintain a horizontal orientation and may have moved by crawling with its tentacles³¹. How strongly their ecologic niche was influenced by tidal interference and if this is reflected in their growth pattern is not known. Thus, specimens with relatively weak septa and/or inability to maintain the body chamber in a horizontal position could not be active deep water swimmers like *Nautilus*. Those fossil specimens of the orders Orthocerida and Nautilida that fit the growth trend are considered to have withstood similar depths as recent *Nautilus*³⁰.

The eyes of *Nautilus*, like those of other cephalopods, are quite sensitive to variations in amount of light, despite the absence in *Nautilus* of iris, lens, and eyelid³². Large numbers of cephalopods migrate upwards at night³³, and *Sepia* becomes so buoyant in darkness that it cannot help but rise³⁴. As for *Nautilus*, its activity has been reported^{21,31}, although not confirmed¹⁹, to be related to light and darkness with the animal becoming active at sunset and migrating nocturnally to lesser depths.

Whether the *Nautilus* secretes growth lines and septa during the day, when it rests in deep water, or at night, when it actively feeds, remains unknown. A theory of molluscan growth line formation based on respiratory changes²² would imply that the growth increments are reflections of alternating periods of shell deposition and dissolution occurring during aerobic and anaerobic respiration, respectively. It might be speculated that for *Nautilus* the aerobic respiration occurs at night in warmer, shallower water, with anaerobic respiration during the day in colder, deeper waters. The issue of daily migratory habits that this latter thesis implies remains at present unresolved^{19,21,31}.

Other astronomical evidence

Other astronomical measurements of changes in the lengths of the day and the lunar distance may be compared to those obtained from our study of nautiloid growth rhythms. The short-term astronomical approach utilises careful measurements of the Moon's position through occultations timed by atomic clocks¹⁵. Although astrometry can determine precisely the rotation rate of the Earth, it cannot give the secular acceleration because, over periods from a few days to several centuries, the acceleration is dominated by non-cumulative fluctuations²⁶. Historical records of ancient solar eclipses¹⁶ provide a means of studying the evolution of the Earth–Moon system for the past 3,000 yr. This approach yields clear evidence that the lunar orbit and the length of the day have increased during this period. The lunar laser ranging experiment can now measure the distance to the Moon with an accuracy of about half of the predicted yearly lunar recession³⁵, and thus will soon be able to provide direct data on changes in the lunar distance.

From the ancient eclipse study¹⁶, the rate of change in the lunar orbit semi-major axis is $\dot{a}/a = 1.5 \times 10^{-10}$ parts yr^{-1} , or a recession of the Moon of 5.8 cm yr^{-1} . The value for the Earth–Moon distance 69.5 Myr ago is 0.840 times the present distance (Fig. 6). This implies an average change in the lunar orbit semi-major axis of 26.6×10^{-10} parts yr^{-1} or an average recession of 94.5 cm yr^{-1} . This is 17 times the rate from the ancient eclipse studies and implies (if this rate is the correct long-term one) that the recent dissipation is not typical for past geologic time and that additional energy sinks may be involved.

The unfortunate problem inherent in the astronomical approach is that there is no way of knowing whether the last few

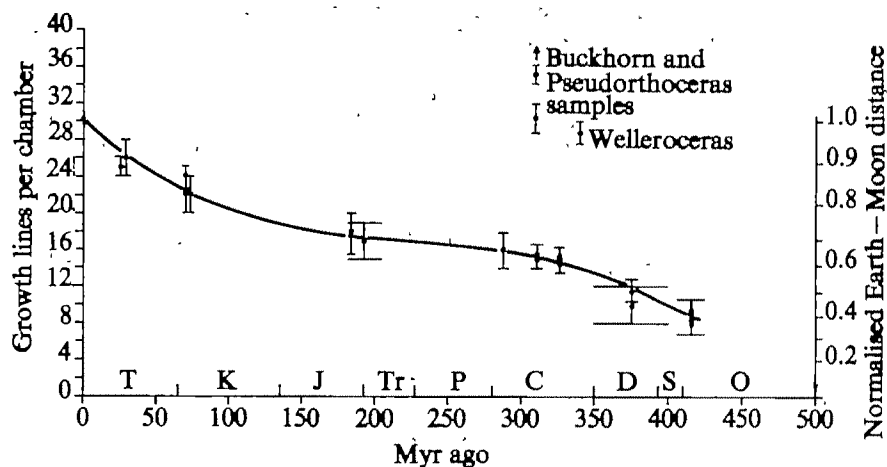
decades of lunar orbital evolution, or even the last few thousand years, are typical of the evolution which occurred over geological times. To extrapolate from a few thousand years to several hundred million is quite risky. Whether the rate of lunar orbital change for the last few thousand years is a 'minor fluctuation' compared to the millions of years of geologic time is not easily determined.

The only 'long-term' study (on a time scale of millions of years) is Wells' palaeontological study¹ on daily and annual banding in corals, the results of which suggest a much slower lunar recession than the nautiloid data does. Although the existence of annual banding on corals is well established, there is some question about daily banding in modern corals. We have seen no research indicating daily bands, except for Wells'

Table 1 Characteristics of available nautiloids

Years ago (millions)	Period	Location	Name	Specimen code	Chambers counted	Average growth lines per chamber	Day length (h)	Synodic month (h)	Sideral month (h)	Earth-Moon distance (present distance = 1.0)	Standard deviation
0	Recent	New Caledonia	<i>Nautilus pompilius</i>	PK 1975A Ocean Museum Natural History	5	29.4±1					
0	Recent	New Caledonia	<i>Nautilus pompilius</i>	PK 1975B Cincin. Museum Natural History	7	29.0±2					
0	Recent	S Pacific	<i>Nautilus pompilius</i>	IA Princeton	4	30.5±2					1.75
0	Recent	S Pacific	<i>Nautilus pompilius</i>	IIB Princeton	2	33.5±2	Average of 30.01 growth lines per chamber				Among recent specimens
0	Recent	S Pacific	<i>Nautilus pompilius</i>	IIIC Colorado	6	29.75±2	24.00	722	667	1.01	
0	Recent	S Pacific	<i>Nautilus pompilius</i>	F Colorado	6	31±2					
0	Recent	S Pacific	<i>Nautilus pompilius</i>	G Colorado	8	30.6±2					
0	Recent	S Pacific	<i>Nautilus pompilius</i>	Bryn Mawr	3	30±1					
0	Recent	S Pacific	<i>Nautilus pompilius</i> (juvenile)	Princeton	2	30±1					
25±4	Miocene	Chipola Formation, Florida	<i>Astris carolinensis</i>	Smithsonian 644819	5	25±1	23.83	596	558	0.898	0.84
28.5±2	Oligocene	'Obg' from Australia	<i>Astris</i> sp.	3262 Princeton	2	26±2	23.80	619	578	0.919	2.83
69.5±1	Upper Cretaceous	Pierre Shale, Wyo., S.D.	<i>Eustrophia deKayi</i>	D1652 USGS Denver	2	22±2	23.52	517	488	0.882	2.83
69.5±4	Upper Cretaceous	Pierre Shale, S. Dakota	<i>Eustrophia deKayi</i>	85219 Princeton	1	22.5±2	23.52	529	499	0.833	0.48
69.5±1	Upper Cretaceous	Pierre Shale, S. Dakota	<i>Eustrophia deKayi</i>	8668 Iowa	3	24±1	23.52	564	530	0.868	1.00
69.5±1	Upper Cretaceous	Pierre Shale, S. Dakota	<i>Eustrophia deKayi</i> (juvenile)	Smithsonian	2	22.5±2	23.52	529	499	0.833	2.12
72.5±2	Upper Cretaceous	Rapley Formation, Coon Creek, Tenn.	<i>Eustrophia deKayi</i>	Smithsonian	2	22±2	23.50	517	488	0.821	2.83
183±3	Lower Jurassic	Charmouth, Dorsetshire, England (LIAS)	<i>Nautilus maritimus</i>	W 4329 Princeton	3	18±2	22.73	409	391	0.708	1.15
183±3	Lower Jurassic	England	<i>Nautilus maritimus</i>	W 5147 Princeton	2	17.5±2	22.73	398	380	0.696	0.71
192±30	Lower Jurassic to Upper Triassic	England	<i>Climacoceras intermedius</i>	W 4331 Princeton	2	17.0±2	22.67	385	369	0.681	0.71
287±4	Upper Pennsylvanian	Orabon Formation, Foss Shale	<i>Brachycycloceras nermale</i>	SUI 609 Iowa	3	16.0±2	22.00	352	339	0.644	0.48
314±4	Middle Pennsylvanian	Ladledale coal	<i>Metacoceras carinatum</i>	+ 36306 Iowa	4	15.0±1	21.82	327	316	0.614	0.82
314±4	Pennsylvanian	Lower Cherokee	<i>Demococeras</i> sp.	+ 3610 Iowa	5	15.5±1	21.82	338	326	0.627	0.55
314±4	Pennsylvanian	Ladledale coal	<i>Tenuechlamis hameri</i>	+ 36311 Iowa	4	15.0±1	21.82	327	316	0.614	0.50
326±2	Upper Mississippian	Imo Formation, Arkansas	<i>Reticocycloceras pyramidalis</i>	SUI 36411 Iowa	15	15.0±1	21.74	326	314	0.613	0.26
326±2	Upper Mississippian	Imo Formation, Arkansas	<i>Reticocycloceras crenatus</i>	SUI 36397 Iowa	8	14.5±0.5	21.74	315	304	0.599	1.26
326±2	Upper Mississippian	Imo formation, Arkansas	<i>Reticocycloceras imoense</i>	W. B. Saunders SUI 36331 Iowa	8	15.37±0	21.74	334	322	0.622	0.00
326±10	Upper Mississippian	Batonville Formation	<i>Orthoceras</i> sp.	W. B. Saunders A, B, C, D (4 specimens)	13 total	15.11±1	21.74	328	317	0.615	0.35
375±25	Lower-Mid Devonian	unknown	unknown	Princeton 681 and W 299	2	10±2	21.40	214	209	0.466	0.21
375±3	Lower-Mid Devonian	Dublin Ohio	<i>Orthoceras</i> sp.	U of Iowa	7 total	11.5±1.5	21.40	246	239	0.511	0.48
420±10	Upper Ordovician	Maquoketa Formation, Iowa	<i>Chonetoceras laeti</i>	U of Iowa	1	8.0±1	21.08	169	165	0.399	0.71
420±10	Upper Ordovician	Oxford Ohio	<i>Chonetoceras beeri</i>	W 4319 A, B Princeton	5	9.0±1	21.08	190	186	0.431	0.71
420±10	Upper Ordovician	Kope Formation near Cincinnati	<i>Thaptoceras</i> sp.	R. Davis Mrs Nat Hist Cincinnati	27	8.5±0.5	21.08	179	176	0.415	0.50
420±10	Upper Ordovician	Graf, Iowa	<i>Dolerthoceras sociale</i>	R. Davis Mrs Nat Hist Cincinnati	3	9.0±1	21.08	190	186	0.431	1.85
420±10	Upper Ordovician	Graf, Iowa	<i>Dolerthoceras sociale</i>	R. Davis Mrs Nat Hist Cincinnati	12	9.5±0.5	21.08	200	196	0.447	0.58

Fig. 6 Plot of the number of growth lines per chamber length and Earth-Moon distance against geologic time showing the tendency towards increase in the number of nautiloid growth lines/chamber with time. The points are fitted by a third degree polynomial least squares fit. Note the consistency with which nautiloids of each era have the same number of growth lines per chamber, with the only exceptions being the three specimens of Carboniferous age, which reflect multiple integrals of the predicted constant number. Independent evidence suggests that these species, in contrast to the other nautiloids studied, lived in a shallow water regime subject to tidal interference. Vertical error bars mark the maximum discrepancy between any single growth line count and the average for the specimen. The horizontal width of each error bar reflects the uncertainty in the dating of each fossil. For changes in the Earth-Moon distance based on the nautiloid data (right scale), the steep slopes in the curve may represent higher tidal energy dissipation during the last 70 Myr as well as from 325–420 Myr ago. About 375 Myr ago the Moon was about one-half its present distance from the Earth.



observation of one coral species in which this seems to occur. Usually incomplete lunar monthly cycles reflect more the coral's environment of growth than lunar periodicity³⁶. Other researchers report that the maximum density of reef coral skeletons is only associated with periods of above average seawater temperature³⁷. Growth patterns vary considerably among modern individual corals, even of the same community³⁸. The potential of corals as geochronometers is seriously limited by the absence of daily and monthly growth patterns in vertical sections of coral skeletons³⁹.

The nautiloid data can provide long-term evidence for the length of the day. By assuming only that the angular momentum of the Earth-Moon system is conserved, the lengths of the day and month are tied inseparably to one another, and, therefore, are both established by the nautiloid data. The past length of the day has not been established by any other growth studies. Calculations on the length of day are being done and will reduce, when substituted into the Runcorn equation, the palaeo-lunar distances in Table 1 and Fig. 6.

Rate of lunar orbital evolution

A synthesis of palaeomagnetic data, stratigraphic relationships, and studies of environments of sediment deposition have led to a reconstruction of Mesozoic and Palaeozoic continental configurations³⁹, however, much more exact and extensive information on ocean profiles is required for even an order of magnitude determination of ancient tidal energy dissipation. Since the magnitude of tidal dissipation depends so strongly on ocean configurations and depths^{40,41}, theoretical extrapolation of the lunar orbit into the past becomes an impossible task⁴¹. Given this limitation, it is still a generally accepted characteristic of lunar orbital evolution that it proceeded most rapidly when the Earth and the Moon were closer together. Tidal forces were then greater because the tidal torque is proportional to $a^{-11/2}$ (ref. 14). This means that the orbit would have quadrupled in size in the first 10 Myr if the Moon originally had been near the Roche limit. However, when the present astronomically determined rate of the Moon's recession is extrapolated back in time, the Moon approaches the Roche limit less than 10^9 yr ago, according to Kaula¹³, and a lunar surface melt would have occurred due to tidal energy dissipation. There is no lunar or terrestrial geologic evidence to support such a close approach at that time, since the youngest rocks on the Moon were crystallised 3.25×10^9 yr ago. This represents a time scale problem which has not been satisfactorily explained.

The change in the Earth-Moon distance shown in Fig. 6 does not proceed at a constant rate, but exhibits rather several slopes, with the slope being most gentle when the continents were not

separated, 225 Myr ago. This may reflect less active mid-ocean ridges, a lower sea level, and thus a corresponding decrease in tidal friction, as well as a decrease in the rate of retreat of the Moon from the Earth⁴²⁻⁴⁴.

The recession rate of the Moon during the last 420 Myr, based on nautiloid data, varies by a factor of less than 3, which does not support a recession rate varying by $a^{-11/2}$. This supports the explanation of the time scale problem in terms of changing palaeogeography. As Kaula¹³ points out, the formation of Pangea at a high latitude would lead to lower tidal dissipation. If tidal dissipation takes place mainly in shallow seas, then a lowering of sea level to a depth below the present day continental shelves could conceivably reduce the tidal dissipation⁴¹. However, the establishment of even non-tidal oceanic circulation patterns in the past is extremely difficult.

If the Moon originally was near the Roche limit about 4×10^9 yr ago, as Lowman⁴⁵ and others have proposed, then the orbit must have evolved slowly for the Moon to be at half of its present distance 375 Myr ago, in the Devonian, as our calculations show. This slower evolution can explain why the lunar maria are concentrated on the near side of the Moon, as well as why the Moon's centre of mass is displaced earthwards relative to its figure. If slower evolution occurred, the Moon may have been close enough to the Earth for gravitational accelerations asymmetric to the centre of the Moon to influence magma extrusion 3.5 to 4.0×10^9 yr ago⁴⁶. Certain aspects of the Witwatersrand sediments, deposited 2×10^9 yr ago, may be explained by larger tides due to a smaller Earth-Moon distance⁴⁷. Studies of Precambrian stromatolites^{48,49} suggested that greater palaeotides existed until 1.7×10^9 yr ago, a feature unexplainable if the Earth-Moon distance increased rapidly early in the system's history⁵⁰. It is difficult to extrapolate beyond the 420 Myr of nautiloid data without an understanding of why the recession does not vary inversely as the $11/2$ power of lunar distance. One must also explain why the recession rate observed in historical times is so much smaller than the long-term average shown by nautiloid data for the past 70 Myr.

A recent summary of geologic evidence on the accretion of continents⁵¹ provides new support for the idea of a lunar orbit slowly expanding from about 4×10^9 yr ago until the Cambrian. One or a few supercontinents may have accreted gradually beneath a globe-encircling ocean in the Precambrian, with the continent(s) emerging only 600 Myr ago, at the beginning of the Cambrian. We, therefore, support the existence of three stages in the dynamical evolution of the Earth-Moon system. First, at a time when liquid water was not present on the surface of the Earth, the lunar orbit evolved slowly due to tides in the solid Earth and Moon. Next, the Moon continued to recede slowly

because the deep sea encircling and covering the continents dissipated little energy. Then, about 600 Myr ago, when the continents emerged, the last stage began, and lunar recession proceeded more rapidly.

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DNA Sequence of the mini-insertion IS2-6 and its relation to the sequence of IS2

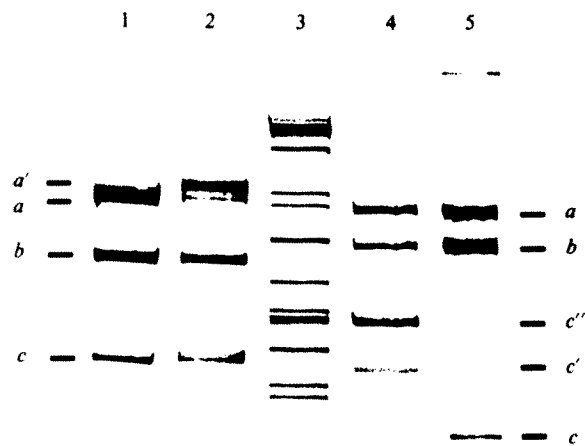
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In polar orientation IS2 abolishes galactose operon expression. Operon activity is restored by a 108 base pair mini-insertion within IS2 called IS2-6. The DNA sequences of the gal operon-IS2 junction, the parental IS2 region undergoing sequence rearrangements and IS2-6 itself are reported. IS2-6 is composed of sequence intervals present in both strands of IS2.

It is known that IS2 occurs in multiple copies in the *E. coli* chromosome¹ and to transpose to various sites in the same or other replicons, at which it sometimes induces mutations². The analysis of such mutations revealed that IS2 can influence the expression of adjacent genes in two different ways depending on its orientation of integration^{3,4}. For example, when integrated into the control region of the galactose operon of *E. coli* in orientation I, IS2 turns off the expression of the *gal* genes downstream in the direction of transcription; in the opposite orientation (orientation II) it efficiently turns them on. We have further shown that an IS2 element integrated in polar orientation (orientation I) can undergo internal changes that also lead to constitutive expression of neighbouring genes, although the gross orientation of the element remains unaltered^{3,5}.

To study the internal changes within IS2 more precisely, a recombinant plasmid, pDG1, was constructed, containing the galactose operon of *E. coli* K12 within the plasmid pSC101. IS2 was then transferred to plasmid pDG1 by homogenote formation, generating the mutation *galOP*-308::IS2. This new plasmid, harbouring IS2 in polar orientation in the control region of the galactose operon is called pDG12. A strain carrying a deletion in the *gal* region of the *E. coli* chromosome was transformed with pDG12 and used for further genetic manipulations⁵. At 37°C Gal⁺ revertants of this strain are observed with a frequency of 3×10^{-7} . Among 109 such Gal⁺ revertants analysed 102 exhibited inducible synthesis of *gal* operon specific enzymes, indicating that the wild-type *gal* operon had been restored, most likely due to accurate excision of the integrated IS2 element. This has been verified for one such inducible revertant⁵. However, seven clones were found to be constitutive for *gal* enzyme synthesis, suggesting that other events had led to reactivation of the *gal* operon. The seven constitutive Gal⁺ revertants fall into three groups: Class 1 consists of one revertant, which was shown by genetic means to be due to a highly unstable suppressor located on the bacterial chromosome. Class 2 contains three revertants which show no gross alterations within IS2, as judged by analysis of DNA fragments produced by various restriction endonucleases. These revertants are stable.



Class 3 consists of three further revertants which are characterised as being highly unstable. These were previously shown to be due to the addition of small DNA segments within IS2. Revertant R1 contains an insertion of 115 base pairs, while in R3 a 65-base pair DNA addition was observed. These mini-insertions were previously termed IS6 and IS7 respectively⁵. We report here the DNA sequence of part of IS2 and of IS6. These results indicate that IS6 can be considered an allele of IS2. Therefore it will henceforth be called IS2-6. The DNA sequence of IS2 and IS2-6 reported here suggests a model for the formation of mini-insertions.

Mapping of IS2-6

In an earlier report the presence of additional DNA within IS2 in two unstable constitutive Gal⁺ revertants of *galOP*-308::IS2 on pDG12 was shown by agarose gel electrophoresis of DNA fragments produced by restriction enzymes⁵. The relevant DNA fragment used in that study was produced by restriction endonuclease *Hind*III.

Fig. 1 Electrophoretic banding patterns of the *Hind*III DNA fragments of pDG12 (IS2) and pDG12 R1 (IS2-6), which are 895 and 1,010 base pairs long respectively⁵, after *Hinf*I and *Hae*III digestion. Slot 1, *Hinf*I digestion of pDG12 DNA, band *a*, *b*, and *c* correspond to 435, 282, and 153 base pairs respectively. Slot 2, digest of pDG12 R1 with *Hinf*I. Note the shift in molecular weight of band *a* (435 base pairs) to *a'* (542 base pairs). The presence of *a* material in pDG12 R1 is due to Gal⁺ segregants present in the DNA preparation. All other bands are unaffected by mutation R1. Slot 3, Marker λ dv-1 digested with restriction endonuclease *Hae*III. From bottom to top the molecular weights of the bands correspond to 120, 136, 150, 180, 185, 210, 240, (286, 331), 451, 481, 1,000, 1,325, and 1,460 base pairs respectively⁸. Slot 4, DNA fragments of pDG12 R1 produced by *Hae*III endonuclease. Bands *a*, *b*, *c''* and *c'* correspond to 430, 340, 207, and 153 base pairs respectively. Slot 5, pDG12 *Hae*III fragments. Bands *a*, *b*, and *c* correspond to 430, 340 and 99 base pairs respectively. Plasmid DNA from pDG12 and pDG12 R1 was prepared as described previously⁵. Preparative isolation of *Hind*III fragments was performed using 6–8 mm thick agarose slab gels (1%); gel strips containing the bands were cut out and DNA was extracted^{9,10}. DNA was pelleted by adding 0.3 M Na-acetate was 2.5 volumes of ethanol. For terminal labelling and further enzymatic cleavage this DNA was purified by one cycle ethidium bromide–CsCl buoyant density gradient centrifugation. Digestion of the DNA (0.5–1.0 μ g) with restriction enzymes *Hinf*I and *Hae*III was performed in 10 mM Tris–HCl buffer pH 7.6, 10 mM MgCl₂, 1 mM DTT, 50 mM NaCl, and 0.1 mM EDTA, and 100 μ g ml^{–1} carboxymethylated BSA. The digest was adjusted to 10 mM EDTA and 10% glycerol, 0.05% bromophenol blue was added, and the mixture was subjected to electrophoresis on 10% polyacrylamide slab gels in 40 mM Tris-acetate buffer pH 8.3 for 2–3 h at 110 V. The gels were stained in water containing 2 μ g ml^{–1} ethidium bromide for 10–15 min. The gel was then photographed under ultraviolet light.

While the *Hind*III fragment generated from plasmid pDG12 is 895 base pairs long, the corresponding fragment produced from DNA presumably carrying IS2-6 (pDG12 R1) is 1,010 base pairs long. When the *Hind*III fragment from pDG12 is further digested with *Hae*III endonuclease, three smaller frag-

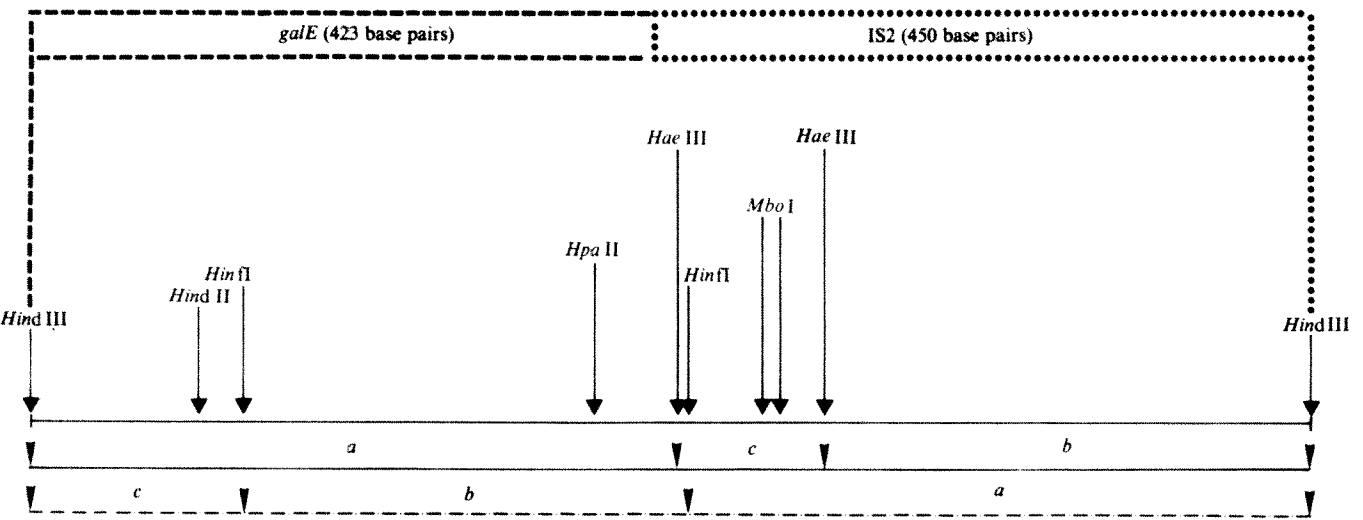
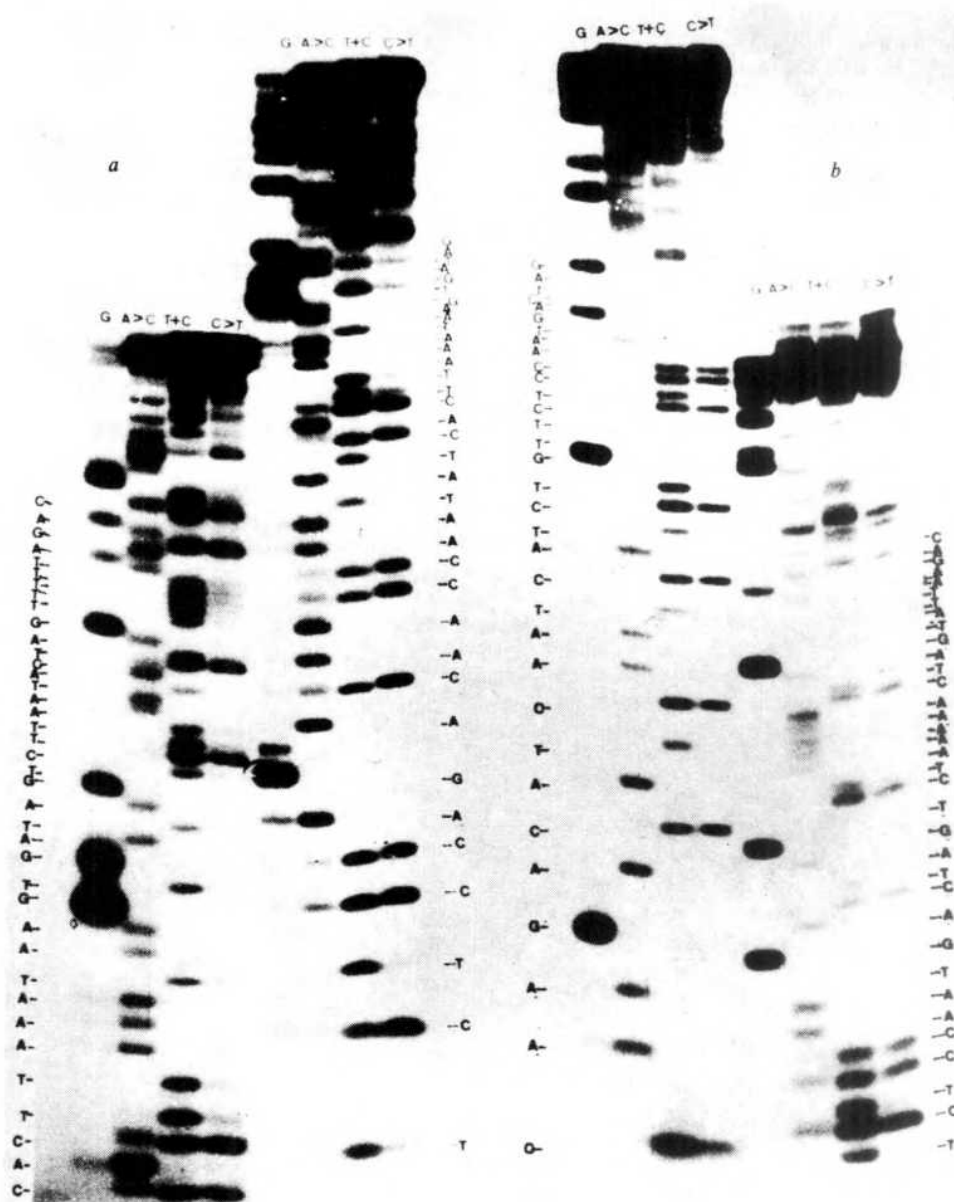


Fig. 2 Detailed restriction map of the 895-base pair⁵ *Hind*III DNA fragment of pDG12. The pertinent sites for cleavage by restriction endonucleases *Hind*III, *Hae*III, and *Hinf*I are shown, including one *Hpa*II site. The *Mbo*I sites are inferred from the sequencing data, but the enzyme does not cleave at these positions (see text). The map was established by digesting the *Hind*III fragment with one enzyme and comparing this with the reciprocal double digest of combinations of various other enzymes. The *Hinf*I and the *Hae*III fragments *a*, *b*, and *c* are given below the map. When terminally labelled *Hind*III fragment of pDG12 is digested with *Hae*III (see Fig. 1, slot 5) fragments *a* and *b* contain the label at one of their 5'-ends, while fragment *c* is not labelled. This shows that fragment *c* is derived from the interior part of the *Hind*III fragment. The position of fragment *a* can readily be identified since it contains the known *Hind*II cleavage site within *galE* (ref. 5). *Hinf*I digestion of the *Hind*III fragment also yields three fragments *a*, *b*, and *c* (see Fig. 1, slot 1). Digestion of *Hae*III fragment *a* with *Hinf*I yields a 153- and a 275-base pair fragment. Digestion of terminally labelled *Hae*III fragment *c* with *Hinf*I generates a 7- and a 92-base pair fragment. This shows that one *Hinf*I cleavage site is located in *galE*, while the other is close to a *Hae*III site within IS2. The only *Hpa*II site shown is generated after digestion of the 282-base pair long *Hinf*I fragment *b* and thus maps at a position already described^{11,12}. The total length of the *Hind*III fragment as determined by the sum of the proper fragments is 873 instead of 895 base pairs as determined previously⁵.

Fig. 3 Autoradiograms of sequencing gels. *a*, [^{32} P]*Hinf*I/*Hae*III fragment from nucleotide 18–74 of IS2. *b*, [^{32} P]*Hae*III/*Hinf*I fragment from 106–52 of IS2. Both sequences read from the labelled 5'-end and represent complementary DNA strands. The sequences overlap at 53–74. About 10–20 pmol of *Hae*III or *Hinf*I fragments of either IS2 or IS2-6 were dephosphorylated with 0.5–1 unit of bacterial alkaline phosphatase (Worthington, phenolysed, ether extracted and ethanol precipitated). Fragments were labelled using [γ - ^{32} P]ATP (1,000–3,000 mCi mmol $^{-1}$, NEN) and 2 units of polynucleotide kinase (Boehringer Mannheim). The doubly labelled fragments were separated on a polyacrylamide gel and the corresponding bands were eluted. These were redigested with either *Hinf*I or *Hae*III to yield a fragment labelled at only one 5'-end. DNA sequencing was performed by partial methylation (for G), controlled partial hydroxymethylation (for C and T) and strong alkaline treatment at 90°C (for A>C) followed by strand scission as described in ref. 6. Products were analysed in 15 or 20% denaturing polyacrylamide gels using Tris-borate-EDTA buffer followed by X-ray autoradiography.



ments (*a*, *b*, and *c*) are generated (Fig. 1, slot 5). However, digestion of the 1,010 base pair long fragment of pDG12 R1 with restriction endonuclease *Hae*III yields three major (*a*, *b*, and *c*), slot 4) and two minor DNA fragments (*c* and *c'*, slot 4). Fragment *c*, not visible in Fig. 1, is identical in size and sequence to the 99 base pair long fragment *c* of pDG12. It is generated by molecules which have lost the mini-insertion within IS2. Frequent spontaneous loss is characteristic of IS2-6. The amount of fragment *c* found in pDG12 R1 preparations correlates with the number of Gal $^{-}$ segregants among cells harbouring plasmid pDG12 R1 (ref. 5).

Fragment *c'* is 153 base pairs long. It is a deletion derivative of IS2-6 and accumulates in cultures with time. Its isolation, properties, and DNA sequence will be reported elsewhere (Ghosal and Saedler, manuscript in preparation).

Two of the three major fragments (*a* and *b*) of the parental and the Gal $^{+}$ constitutive revertant plasmid DNA pDG12 R1, are identical in size. Fragment *c* of pDG12 is 99 base pairs long while the corresponding fragment of pDG12 R1, fragment *c'*, is 207 base pairs long. The mini-insertion IS2-6 is therefore 108 base pairs long and has arisen by insertion within the small

*Hae*III fragment *c* of pDG12, yielding fragment *c''* of pDG12 R1. Both fragments, *c* and *c''*, (Fig. 1) have been used for DNA sequence analysis.

The location of *Hae*III fragment *c* within IS2 and with respect to the most proximal *gal* structural gene is shown on the restriction map of the 895-base pair long *Hind*III fragment of pDG12 presented in Fig. 2.

DNA sequence of part of IS2

The DNA sequence between the *Hpa*II site and the rightmost *Hae*III cleavage site on pDG12 (Fig. 2) was determined using the procedure described by Maxam and Gilbert⁶.

In one set of experiments the *Hind*III fragment of pDG12 carrying *galOP*-308::IS2 was isolated and digested with *Hae*III endonuclease. The mixture of three smaller fragments which results was dephosphorylated and the 5'-ends were labelled with [γ - ^{32} P]ATP using polynucleotide kinase. Fragment *c* (see Fig. 1) was isolated and its labelled strands separated by gel electrophoresis. These were subsequently sequenced.

In an alternative procedure, the labelled *Hae*III fragment *c* was further digested with *Hinf*I. The larger 92-base pair frag-

ment labelled at its *Hae*III end was isolated and sequenced. For determination of the sequence of the complementary DNA strand, the *Hind*III fragment was first cleaved with *Hinf*I. The mixture of the three fragments generated (see Fig. 1) was labelled and subsequently digested with *Hae*III. In this case the 92-base pair fragment is labelled at its *Hinf*I end. This fragment was isolated and sequenced.

The sequencing gels of the latter two fragments are presented in Fig. 3*a* and *b*. The sequences obtained with both procedures are identical except for 7 base pairs to the left of the *Hinf*I cleavage site, which are missing in the *Hinf*I-*Hae*III fragments. The complete sequence of the *Hae*III fragment *c* is included in Fig. 5.

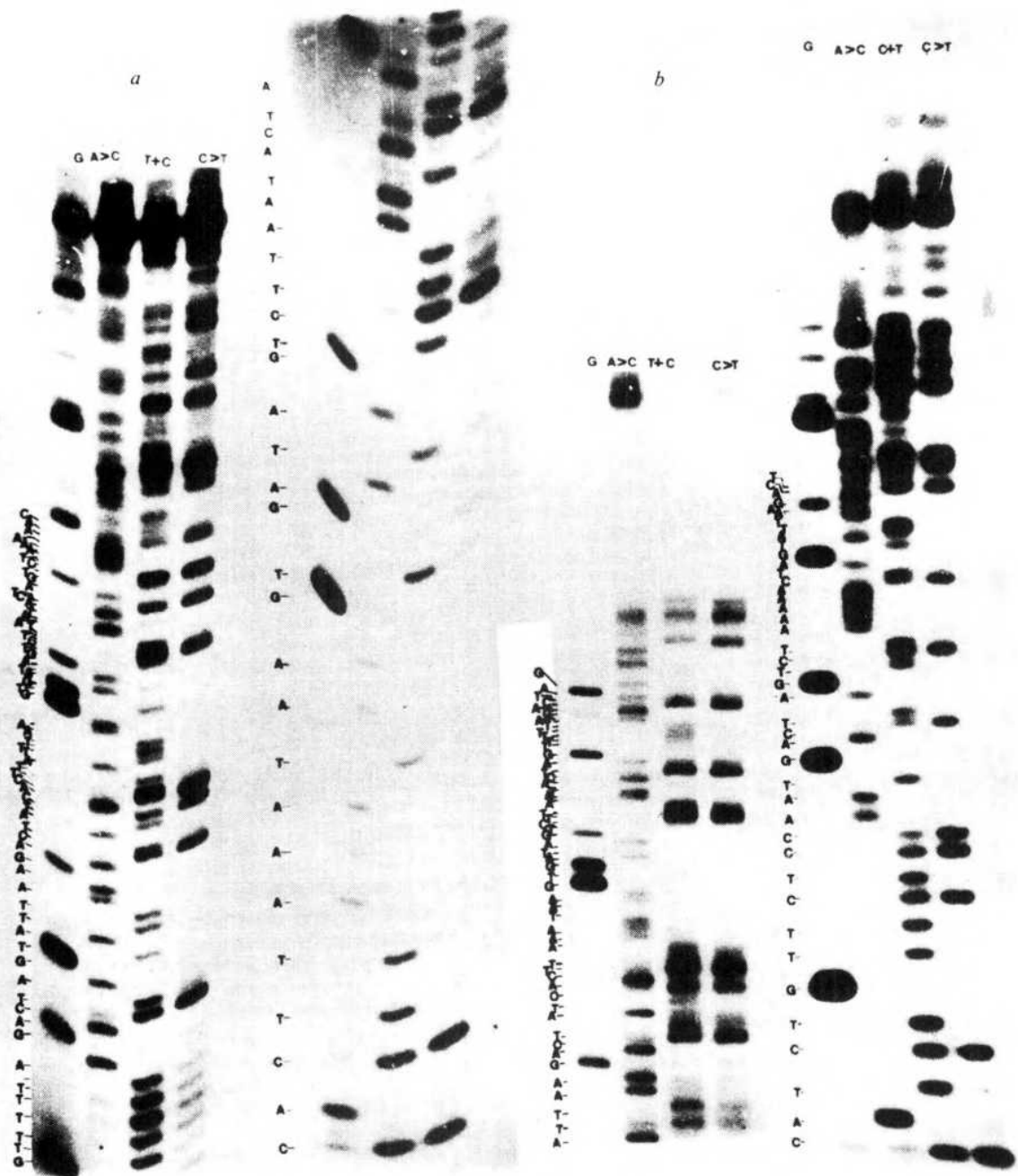
Two *Mbo*I sites are detected by the sequencing method. However, *Mbo*I does not cleave at these positions. This seems to

indicate that the *Mbo*I sites are modified in this bacterial background. This agrees with observations by others that the *Mbo*I cleavage site is modified in *E. coli* K12.

Integration site of IS2

On cleavage with *Hinf*I the *Hind*III fragment yields three smaller fragments as shown in Fig. 1, slot 1. The 282-base pair fragment *b* contains the junction between IS2 and the *gal* operon. Labelling both 5'-ends and subsequent digestion with *Hpa*II yielded a fragment suitable for sequencing. The DNA sequence of the integration site of IS2, which also contains the *gal* mRNA leader sequence and the total sequence of the *Hae*III fragment *c* is included in Fig. 5. It can be seen that IS2 has integrated next to the initiation site for *gal* mRNA tran-

Fig. 4 IS2-6 was sequenced by the methods described in the legend to Fig. 3. *a*, DNA sequence of the labelled IS2-6 *Hinf*I fragment *a'* cleaved with *Hae*III, from position 36-128. *b*, DNA sequence of the complementary DNA strand of IS2-6 from 201-116 (labelled *Hae*III fragment *c''*, cleaved with *Hinf*I). The overlapping region is thus 13 base pairs long.



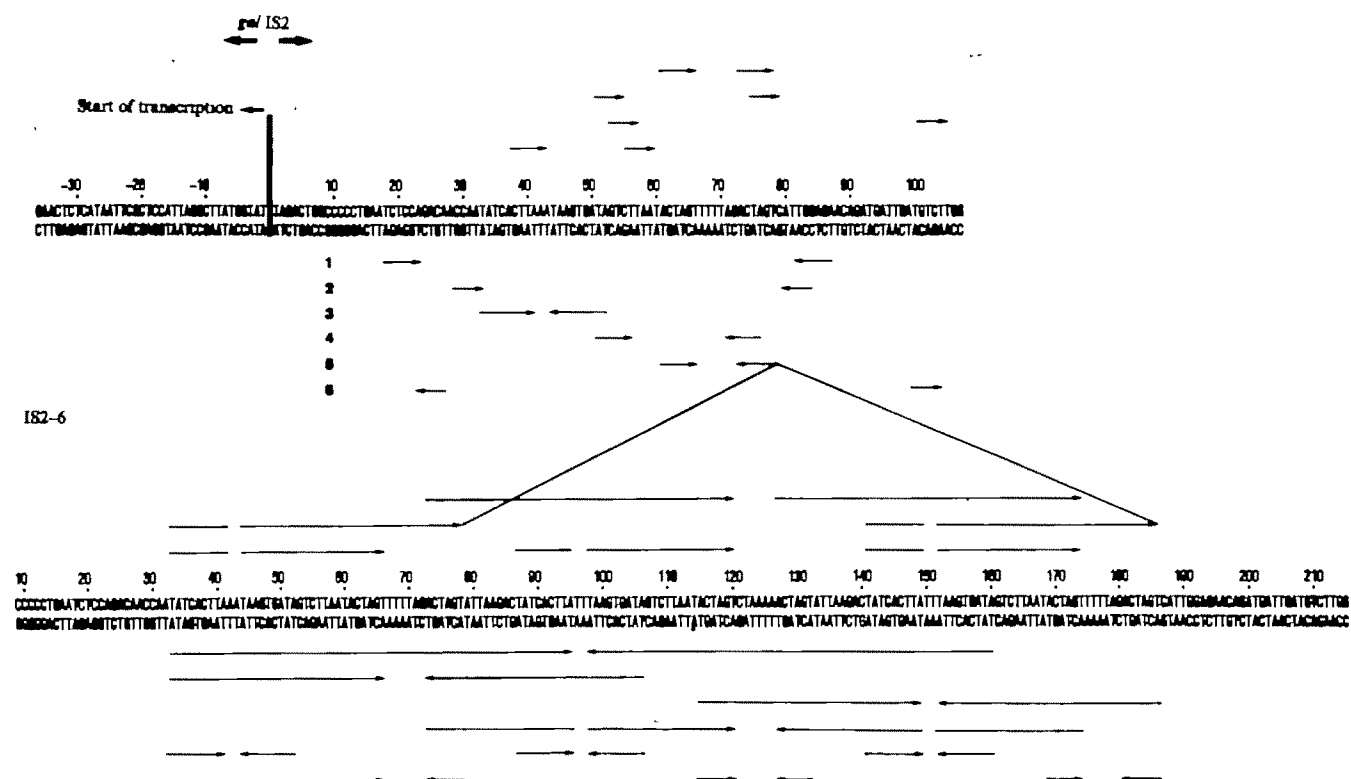


Fig. 5 Sequences with negative numbers represent material from the galactose operon, others are IS2 and IS2-6 sequences, respectively. The vertical bar in the top line separates the *gal* from the IS2 sequences. The arrows above the sequences indicate direct sequence repetitions, while the arrows below the sequences show the inverted repeats. For IS2 the small inverted repeats are numbered from top to bottom. The connecting lines between IS2 and IS2-6 show the length and the position of the 108-base pair insert of IS2-6.

scription. Thus in mutation *galOP*-308, IS2 disconnects the mRNA initiation site from the *gal* promoter. Comparison with the sequence of the operator proximal end of IS2 reported for *galOP*-490::IS2 (ref. 7) reveals that in both strains IS2 has integrated into the control region of the *gal* operon, but at two different sites, separated by one nucleotide. In addition the comparison also reveals that the termini of IS2 correspond to imperfect inverted repeats.

Features of the IS2 sequence

Figure 5 also shows that there are a few small direct and inverted repeats in the sequence of the *Hae*III fragment *c* of IS2. Six pairs of inverted repeats are present. It should be noted that the sequence between the left end of pair 3 and the right end of pair 5, that is, nucleotide pair 33 to nucleotide pair 78, is especially rich in A-T (76%), while the A-T content of the flanking sequences is about 50%.

Features of the sequence of IS2-6

The same strategy has been used to analyse IS2-6, the *Hae*III fragment containing IS2-6, being 207 instead of 99 base pairs long.

The sequencing gels for the *Hinf*II/*Hae*III fragments are given in Fig. 4a and b. The DNA sequence obtained is included in Fig. 5. Except for the modified *Mbo*I sites already seen in the IS2 sequence, no other new cleavage sites for restriction endonucleases are found in IS2-6.

The most striking feature of the IS2-6 sequence is the high degree of symmetry. The large inverted repeat is 62 base pairs long and is substructured as indicated. In addition there is large perfect duplication of 46 base pairs in length as well as five smaller imperfect direct repeats. The A-T content between positions 33 and 186 is 76%.

Comparison of IS2 and IS2-6

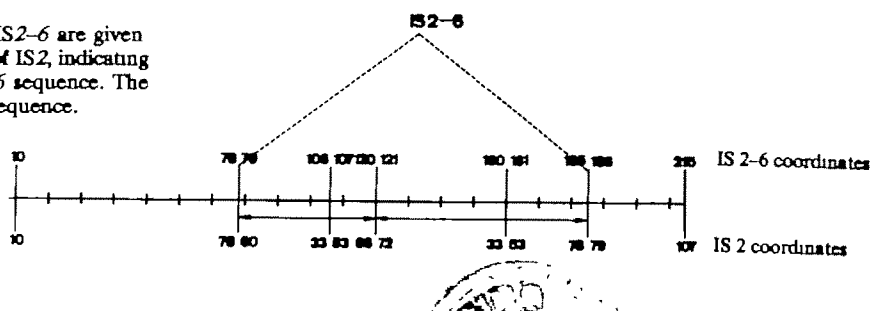
Figure 5 shows that IS2-6 constitutes an insertion of 108 base pairs within IS2 at either position 78/79 or position 44/45. Assuming position 78/79 to be the point of DNA addition, it is clear that the 108 base pairs of IS2-6 are derived from sequences of IS2 located between positions 33 and 78, that is, from IS2 material contained between the inverted repeat pairs 3 and 5.

However, IS2-6 does not consist of a simple duplication of IS2 sequences from these regions. Instead sequences of both IS2 strands located within the 33-78 interval are multiplied and rearranged in the manner outlined in Fig. 6. Some IS2 sequences are represented several times in both possible orientations in IS2-6. The 53-60 region of IS2, for example, recurs five times, while the pairs of inverted repeats 3 and 5 are found three times and in both orientations.

Mode of formation of IS2-6

One explanation of how the complicated structure outlined in Fig. 6 might have arisen is that recombinational events occurred between the two DNA strands of IS2 during replication.

Fig. 6 The coordinates of one DNA strand of IS2-6 are given. The numbers below the line are the coordinates of IS2, indicating which sequences of IS2 are present in the IS2-6 sequence. The arrows emphasise the orientation of the sequence.



However, the high degree of symmetry observed in IS2-6 suggests another mechanism of formation based exclusively on replication. This mode of formation will be described briefly (Fig. 7) as it enables predictions to be made about the sequences of other replication products that would be expected to occur.

Considering the replication of the lower DNA strand of IS2 only, the DNA synthesising enzyme may be imagined to proceed from the 5'-end across the inverted repeat at position 33/52 (pair number 3) to position 78 (Fig. 7a). Because of the high A-T content of this region, partial melting might occasionally

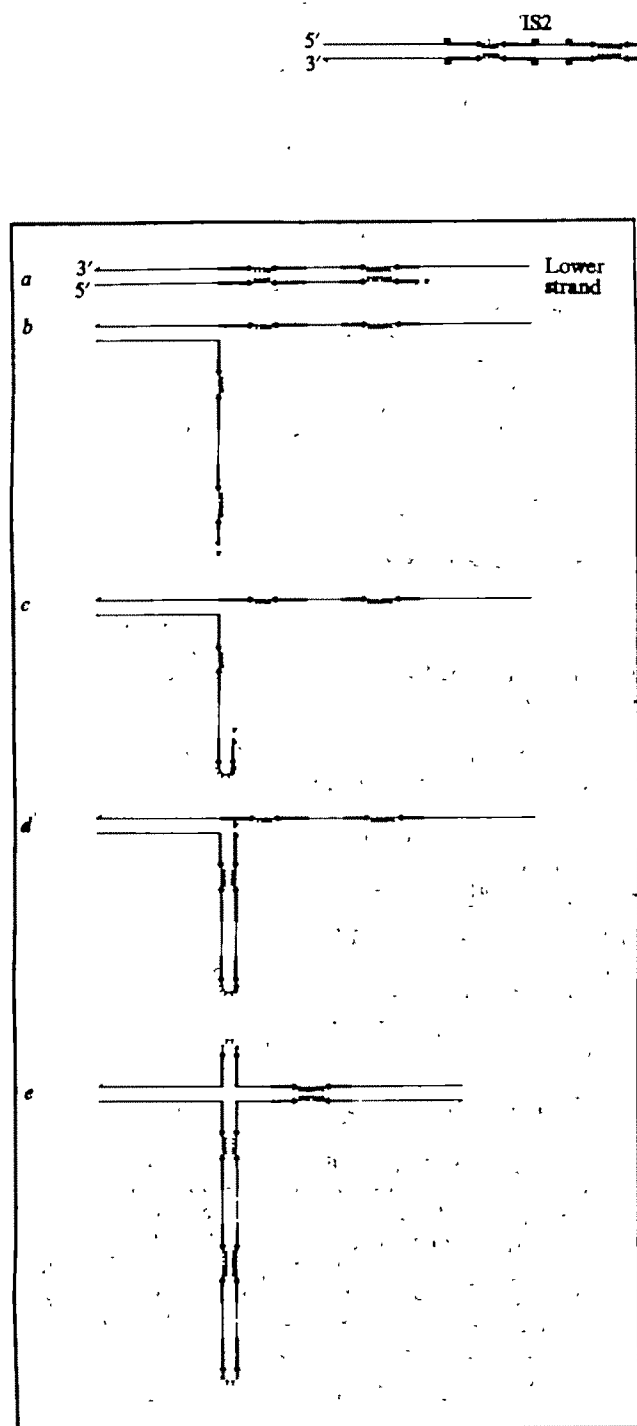


Fig. 7 Slippage replication of the lower DNA strand of IS2 (upper frame) indicate the positions of the inverted repeat pairs 3 and 5 (see Fig. 5), which are marked by heavy arrows throughout. The hypothetical sequence of events (a-e) leading to the replication products IS2 and IS2-6 are enclosed by a black line. For simplicity only the DNA sequences separating both parts of an inverted repeat pair are shown. e, dotted and dashed lines indicate DNA synthesised after a second round of slippage of the DNA template. The segregation products of the heteroduplex formed are presented in the lower frame as IS2 and IS2-6. See text for details.

occur (Fig. 7b). Subsequently inverted repeat pair 5 of the newly synthesised DNA strand could fold back on itself forming a new template for further DNA synthesis (Fig. 7c). The polymerase could then proceed in the 3' direction along the newly synthesised strand until it passes inverted repeat pair 3 and reaches position 33 (Fig. 7d). At this point the same slippage process is repeated: melting out, folding back of the inverted repeat pair 3 and DNA synthesis along the newly made DNA strand leading to the structure shown in Fig. 7e. This slippage tends to form long inverted repeats. Occasionally these may resist partial melting out so that DNA synthesis can then proceed along the old parental DNA strand, provided that inverted repeat pair 3 (33–52) in the parental DNA strand has folded up as indicated in Fig. 7e to yield a stable structure.

On another round of replication the DNA heteroduplex molecule forms two segregants, the old parental IS2 sequence and the newly generated IS2–6 allele.

The slippage of the DNA template during replication of IS2 as outlined in Fig. 7 generates symmetrical DNA additions (see Fig. 5). The process requires, first, a DNA region rich in A–T, and second, pairs of inverted repeat sequences at both ends of the A–T rich region. The newly formed sequences generated by this process may contain new signals for the turn-on of gene expression, as shown by IS2–6. Which parts of the IS2–6 sequences constitute the turn-on signal is not yet known.

The replication 'slippage' model described in Fig. 7 for IS2–6 allows us to predict the DNA sequence of the replication 'slippage' product of the upper DNA strand of IS2. Since preparation of this manuscript we have determined the sequence of the 54-base pair mini-insertion IS2–7. Its sequence is identical to the slippage product predicted for replication of the upper strand of IS2 and will be reported elsewhere¹³.

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Phenotypic expression in *E. coli* of a DNA sequence coding for mouse dihydrofolate reductase

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The construction and analysis of bacterial plasmids that contain and phenotypically express a mammalian genetic sequence are described. Such plasmids specify a protein that has enzymatic properties, immunological reactivity and molecular size characteristic of the mouse dihydrofolate reductase, and render host cells resistant to the anti-metabolic drug trimethoprim.

SINCE the initial propagation of eukaryotic DNA in bacteria¹, several systems have been used to study the expression in *Escherichia coli* of DNA derived from higher organisms. Biological activity of genes from the lower eukaryotes, *Saccharomyces cerevisiae*^{2,3} and *Neurospora crassa*⁴, has been demonstrated using phenotypic selection for functions that complement mutationally inactivated homologous bacterial genes. Immunological reactivity with antibody made against human somatostatin was shown for a peptide fragment cleaved *in vitro* from a hybrid protein encoded in part by bacterial DNA and in part by a chemically synthesised somatostatin DNA

sequence⁵. Very recently, a protein containing amino acids of rat proinsulin was shown to be made by bacteria that carry a double-stranded complementary DNA (cDNA) transcript of pre-proinsulin mRNA⁶; in that instance, antigenic determinants for both insulin and the bacterial enzyme β -lactamase were detected on a fused peptide transported outside the cell. It is not known, however, whether the mammalian peptide components of such immunologically reactive hybrid proteins have functional biological activity.

Our approach to the study of mammalian gene expression in bacteria has been to generate a heterogeneous population of clones carrying a DNA sequence that codes for a selectable mammalian gene product, and then to select directly those bacteria in the population that phenotypically express the genetic sequence. The mammalian enzyme dihydrofolate reductase (DHFR), which catalyses the conversion of dihydrofolic acid to tetrahydrofolic acid, is especially suitable for this purpose. The mammalian DHFR has a much lower affinity for the antimetabolic drug, trimethoprim (Tp), than does the corresponding bacterial enzyme⁷. Thus, bacteria which biologically express mammalian DHFR activity are resistant to levels of trimethoprim that ordinarily inhibit growth.

When these studies were initiated, the only bacterial host approved for EK2 recombinant DNA experiments⁸ was *E. coli* K12 strain χ 1776 (ref. 9). As this strain is already resistant to

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high concentrations of trimethoprim because of its *thy*⁻ mutation¹⁰, direct selection of bacteria that synthesise the mammalian DHFR could not be carried out. Therefore, our initial studies used an *in situ* hybridisation procedure¹¹ to identify χ 1776 clones which carried a mouse DHFR cDNA sequence, with the expectation that a highly sensitive indirect radioimmunoassay¹² would be used to detect any clones that expressed antigenic determinants of the protein product encoded by the gene.

Construction and cloning of chimaeric plasmids containing a DHFR ds cDNA

Figure 1 summarises the experimental scheme used in this study. Partially purified mRNA containing DHFR sequences from methotrexate-resistant AT-3000 mouse cells¹³ was used in the preparation of double-strand (ds) cDNA by RNA-dependent DNA polymerase (reverse transcriptase) and DNA polymerase I (ref. 14 and Fig. 2). Homopolymeric deoxy-C 'tails' were added to the unfractionated cDNA by terminal deoxynucleotidyl transferase (Fig. 2) and the section of the gel containing the predominant (1,500 base pairs) tailed cDNA was eluted. The material recovered from the gel was annealed with an equimolar concentration of pBR322 plasmid DNA that had been cleaved in the β -lactamase gene by the *Pst*I endonuclease¹⁵ and treated with terminal transferase to add homopolymeric dG tails at the cleavage sites. The extent of annealing of DHFR ds cDNA with the plasmid vector was monitored by electron microscopy¹⁶ using circle formation as an indicator. *Pst*I sites are regenerated at both ends of the insert as a result of such recircularisation^{17,18}.

Constructed chimaeric plasmids were introduced into MnCl₂-treated¹⁹ *E. coli* K12 strain χ 1776 and tetracycline (Tc)-resistant transformants (yield ~30 colonies per ng DNA) were selected and tested separately for the presence of a DNA species complementary to a highly purified DHFR cDNA probe. About 40% of the Tc-resistant clones gave a positive reaction by an *in situ* hybridisation test¹¹ (Table 1 and Fig. 3). Plasmids from 14 of the reacting clones were examined by gel electrophoresis and all were found to contain a single DNA insert approximately 1,500 base pairs in length. As both ends of the *Pst*I-cleaved pBR322 DNA receive homopolymeric dG tails, only those molecules that have acquired a dE-tailed cDNA insert or had escaped cleavage and/or dG tailing would be expected to re-circularise and transform; thus, we presume that most of the nonreacting

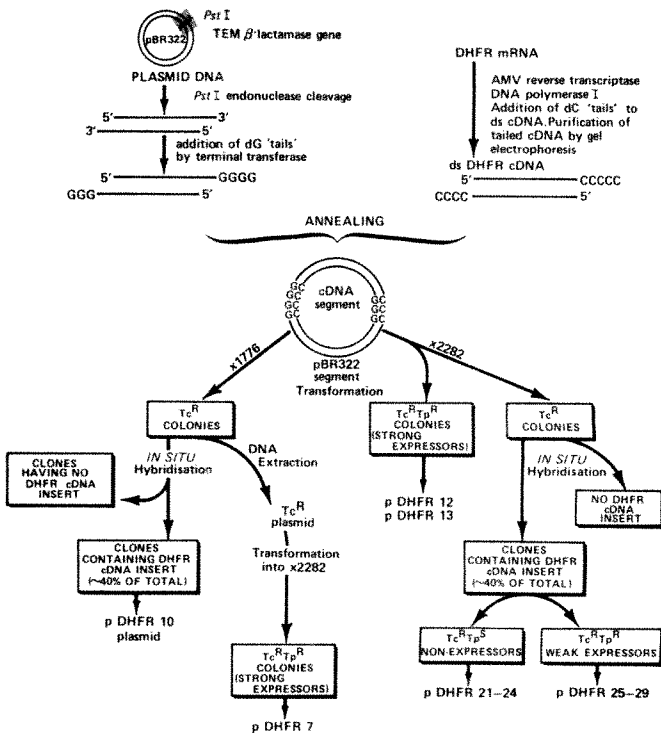


Fig. 1 Scheme used for cloning and expression of mouse DNA sequences that code for DHFR. Additional experimental details are given in the legend to Table 1 and in the text.

Tc-resistant colonies contain inserts of contaminating non-DHFR cDNA.

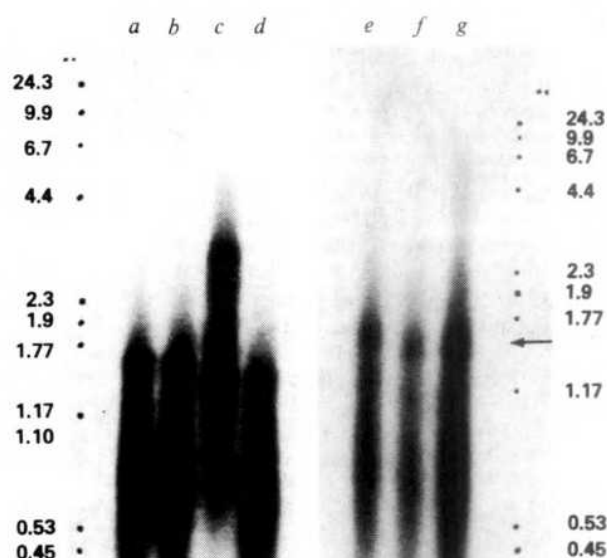
When χ 2282, a *thy*⁺ variant of χ 1776, was approved for EK2 use, direct selection of bacteria that phenotypically expressed the eukaryotic DNA sequence became feasible (see Fig. 1). Two populations (25 colonies each) of previously identified Tc-resistant colonies of χ 1776 were pooled, and plasmid DNA extracted from these populations was introduced by transformation into χ 2282. In other experiments, we transformed χ 2282 directly with annealed pBR322-DHFR cDNA (Table 1). Colonies that expressed resistance to both Tc and Tp were obtained in both types of experiments; three independent

Table 1 Transformation experiments using χ 1776 and χ 2282

Host	Transforming DNA		Transformants per ng DNA			<i>In situ</i> hybridisation with DHFR cDNA probe (% positive)
	Plasmid vector	Insert	Tc (5 μ g ml ⁻¹)	Tc (10 μ g ml ⁻¹)	Tc/Tp (5 μ g ml ⁻¹ of each)	
χ 1776	pBR322	None	4 $\times$ 10 ²	2 $\times$ 10 ²	<2 $\times$ 10 ⁻³	—
χ 1776	pBR322	cDNA(1°)	60	32	—	40
χ 2282	pBR322	cDNA(1°)	70	—	2	44
χ 2282	pBR322	cDNA(2°)	60	—	1.3 $\times$ 10 ⁻¹	—
χ 2282	pDHFR7	—	75	—	25	—

pBR322 plasmid DNA that had been annealed *in vitro* with dC-tailed DHFR cDNA (designated 1°) was introduced into χ 1776 or χ 2282, using a modification of a previously described transfection procedure¹⁹. 1 ml of an overnight bacterial culture was inoculated into 100 ml of L broth supplemented with diaminopimelic acid (DAP, 50 μ g ml⁻¹) and (for χ 1776 only) thymidine (4 μ g ml⁻¹). Bacterial cultures were grown until exponential phase at 35°C and then collected by centrifugation at 4°C. Cells were washed in 0.3 volume 10 mM NaCl, resuspended in 30 ml freshly prepared MCN buffer (70 mM MgCl₂; 40 mM sodium acetate, pH 5.6, and 30 mM calcium chloride) and chilled on ice for 20 min. Cells were collected, resuspended in 1 ml MCN and added in 200- μ l aliquots to 50 μ l DNA in TEN (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA, 50 mM NaCl) or MCN buffer. After chilling at 0°C for 30 min, reactions were incubated at 27°C for 5 min, chilled again for 30 min, and 50- μ l samples were plated on to Penassay broth agar supplemented with DAP, thymidine (for χ 1776), and antibiotics as indicated. When χ 2282 was used, the selective medium was M9 minimal agar supplemented with 0.5% casamino acids, biotin (2 μ g ml⁻¹), DAP (50 μ g ml⁻¹) and Tp (2.5–10 μ g ml⁻¹) plus tetracycline (Yc) or kanamycin (Km) as indicated. Plates containing transformants were incubated at 32°C and colonies were scored 2–3 d after plating. pBR322 plasmid DNA lacking the cDNA insert was used as a control. cDNA preparations labelled as 2° consisted of plasmid DNA isolated from a nonfractionated population of clones that had previously been transformed with chimaeric molecules carrying a cDNA insert. In the experiment shown in the last line the transforming DNA was isolated from a clone (pDHFR7) that expresses resistance to Tp as well as Tc.

Fig. 2 Preparation and characterisation of DHFR cDNA insert. DNA complementary to DHFR mRNA was synthesised essentially as described elsewhere¹⁴, using avian myeloblastosis virus (AMV) reverse transcriptase and polysomal RNA obtained by indirect immunoprecipitation of DHFR-synthesising polysomes from methotrexate-resistant AT-3000 S-180 mouse cells¹³. The RNA had been estimated to contain DHFR mRNA as 20% of its mRNA¹³. The reaction was carried out in 100 μ l using 340 μ g polysomal RNA (estimated to contain 5 μ g polyA-RNA), 45 units AMV reverse transcriptase and dCTP labelled to 4 Ci mmol⁻¹ with ³²P-dCTP (Amersham). Approximately 1.4 μ g cDNA was synthesised in 30 min. The reaction was stopped by the addition of EDTA (to 10 mM) and extracted with phenol, followed by ether, before being passed over a Sephadex G-50 fine column in 10 mM Tris, pH 7.4, 2 mM EDTA and, 10 mM NaCl (TEN). The void volume was collected and precipitated with ethanol. After centrifugation, the RNA was hydrolysed with NaOH (ref. 31), neutralised and precipitated with ethanol. The cDNA was then used as template for the synthesis of the second strand by *E. coli* DNA polymerase I essentially as described³¹. The reaction took place at 42 °C for 10 min in 100 μ l using 1.1 μ g cDNA, 10 units DNA polymerase I, and 200 μ M of each deoxynucleotide triphosphate with dCTP adjusted to 30 Ci mmol⁻¹ as above. Approximately 0.85 μ g of the second strand was synthesised. The reaction was stopped and extracted as above before being passed over a Sephadex G-50 fine column in TEN containing only 0.1 mM EDTA. Column fractions containing the ds cDNA were then treated with *Aspergillus oryzae* S₁ nuclease as described elsewhere³¹. After extraction and precipitation with ethanol, approximately 1.0 μ g ds cDNA was obtained. Aliquots of *a*, first strand product; *b*, first strand product after base treatment; *c*, second strand product and *d*, second strand product after S₁ nuclease treatment were examined on a 1.5% agarose gel in alkaline conditions as described³². Terminal addition of dCTP to the ds cDNA by terminal deoxynucleotidyl transferase (TdT), prepared as described elsewhere³³ was carried out by a modification³⁴ of the Co²⁺ procedure³⁵. The reaction was carried out in 500 μ l containing 140 mM cacodylic acid, 30 mM Tris base, 110 mM KOH (final pH 7.6), 0.1 mM dithiothreitol, 150 μ M dCTP (adjusted to 8 Ci mmol⁻¹ with ³H-dCTP (Amersham)), 1 mM CoCl₂ (added to prewarmed reaction mix before enzyme addition), approximately 1.0 μ g ds cDNA (assuming an average MW giving approximately 600 base pairs, this provides 10 pM 3' termini per ml) and 0.5 μ l TdT (2.3 $\times$ 10⁵ units ml⁻¹). The reaction was allowed to proceed at 37 °C for 10 min before being cooled and sampled to determine incorporation. Approximately 30 dC residues were added per 3' terminus. The reaction was stopped, extracted, desalted and precipitated with ethanol as above. Aliquots of *e*, second strand product; *f*, second strand product after S₁ nuclease treatment, and *g*, dC-tailed ds cDNA were analysed on a 1.7% agarose gel in Tris-acetate-NaCl (ref. 36). The dC-tailed ds cDNA was then preparatively electrophoresed on a similar gel and the '1,500-base pair' region (arrow) cut out of the gel and electrophoretically eluted into a dialysis bag as described elsewhere³⁷. The eluted material was extracted as above, concentrated by lyophilisation and precipitated with ethanol. After centrifugation, the 1,500-base pair dC-tailed ds cDNA (approximately 80 ng) was redissolved in 10 mM Tris HCl, pH 7.4, 0.25 mM EDTA and 100 mM NaCl (annealing buffer). pBR322 plasmid DNA, isolated as described elsewhere³⁸ was digested with a 1.5-fold excess of *Pst*I endonuclease in conditions suggested by the vendor (New England Biolabs) and the linear plasmid DNA was cut out and eluted from a 0.7% agarose gel in TBE³⁹ as described above. The plasmid DNA was 'tailed' with dG residues using procedures similar to those described above. Approximately 15–20 dG residues were added per 3' terminus. Following extraction, the dG-tailed vector was passed over a Sephadex G-50 fine column in annealing buffer and the void volume was collected. Equimolar amounts of dC-tailed ds cDNA and dG-tailed vector DNA were allowed to anneal essentially as described by W. Rowe and R. A. Fratell (personal communication) except that the vector concentration was kept at 75 ng ml⁻¹ in the annealing reaction. Circularisation was monitored by electron microscopy¹⁶ and was typically about 20–40%. This annealed DNA was used directly for transformation into χ 1776 or χ 2282.



transformants were found to be capable of immediate growth in media containing at least 1,000 μ g ml⁻¹ of Tp, and were termed 'strong expressors'. Plasmid DNA isolated from these colonies was shown by repeat transformation to encode both the Tc and Tp resistance phenotypes. However, Tp^RTc^R transformants occurred in different experiments at only 20–60% of the frequency observed when selection of transformants was carried out for Tc resistance alone. Transfer of Tc-selected colonies on to minimal medium agar plates containing Tp showed that all such clones express resistance to at least 1,000 μ g ml⁻¹. Together, these findings suggest that phenotypic expression of Tp resistance may be delayed in transformants until a sufficient quantity of plasmid-specified DHFR has accumulated. Analogous results have been obtained with other antimicrobial drug-resistance determinants encoded by plasmids introduced into *E. coli* by transformation²⁰.

Structure of the DHFR cDNA

Gel electrophoresis of endonuclease-cleaved plasmid DNA from three separately derived bacterial clones that expressed high levels of trimethoprim resistance showed similar overall patterns. Using such data, a cleavage map (Fig. 4) of the cDNA insert of one of these (pDHFR7) was constructed.

Examination of the amino acid sequence of the mouse DHFR enzyme²¹ allowed us to assign one of the *Hae*III cleavage sites to the *trp-pro* (TGG-CCX) present at amino acid positions 24 and 25, thus localising the DHFR structural sequence to a position near the 5' end of the mRNA template used in synthesising the cloned cDNA. Other cleavage sites within the DHFR structural sequence were consistent with positions predicted by computer analysis of the amino acid sequence. DNA sequence analysis of two separate regions of the cDNA provided direct verification that the nucleotide sequence of the insert corresponds to the amino acid sequence reported for the mouse DHFR enzyme, and also confirmed that the coding sequence for DHFR is located at the ds cDNA equivalent of the 5' end of the mRNA.

The nucleotide sequence at the pBR322-cDNA junction nearest the 5' end of the mRNA used as template for DHFR cDNA (that is, at the *Pst*I₀ site) is of special interest. The complement of the 'sense' strand of β -lactamase gene of the vector (J. G. Sutcliffe, personal communication) is interrupted at the *Pst*I site by a series of 11 dG residues added by the terminal transferase, and these are followed immediately by (1) an ATG (AUG) protein start codon, and (2) the codon for the first amino acid of the mouse DHFR structural gene. The sequence that codes for the mouse DHFR is in the same orientation as that encoding the β -lactamase on the vector plasmid; however, the

number of incremental G residues (that is, 10) at the vector-insert junction ensures that the DHFR cDNA sequence is not in the same translational reading frame as the β -lactamase gene.

If the phenotypic expression we have observed for the mouse DHFR sequences in bacteria is the result of translational readthrough from signals that initiate protein chains within the β -lactamase gene, then the host bacterial cell must be able to circumvent the observed frame shift by a 'slippage' mechanism of translation. Such an event might potentially be aided by the long run of dG residues introduced at the pBR322-cDNA junction and could account for our observation (Table 1) that plasmids containing a DHFR cDNA insert yield more than twice as many expressors as would be expected from considerations of reading frame and orientation. However, the high level of functional expression observed for both primary and secondarily transformed clones of pDHFR7 does not seem to be readily explained by slippage of tRNA molecules during translation.

Perhaps a more likely explanation is that the *Pst*I-polyG-ATG sequence that has been constructed preceding the coding sequence for DHFR serves as a binding and protein initiation site for the bacterial ribosome. Recent studies^{22,23} have identified sequences on mRNA in the 5' direction from the initiator codon that are complementary to the CCUCC sequence at the 3' end of the 16S ribosomal RNA species, proposed by Shine and Dalgarno²⁴ to be involved in the binding of mRNA to ribosomes. It is tempting to speculate that the mRNA transcript from the sequence at the pBR322-cDNA

junction has sufficient complementarity to the CCUCC sequence to allow ribosomal binding when a translational start signal is located an appropriate distance away. In such an event, the ATG protein start signal that immediately precedes the coding sequence for the mouse DHFR might initiate a peptide chain having a size characteristic of the mammalian enzyme. Immunological analysis of extracts derived from pDHFR7 and other expressing clones has yielded results consistent with this interpretation (see below).

Analysis of enzyme activity encoded by pDHFR7 plasmid

Mammalian dihydrofolate reductases can be distinguished from their bacterial counterparts by the ability of mammalian enzymes to use folate as a substrate and by their differential sensitivity to competitive inhibitors^{25,26}. In initial experiments, the reduction of folate to tetrahydrofolate was measured using extracts from the pDHFR7 clone, from a Tp-sensitive clone containing a DHFR cDNA insert (the pDHFR10 plasmid), and from cells that contain only the pBR322 vector. Although all three clones are capable of synthesising a chromosomally produced bacterial enzyme, only the enzyme present in extracts from cells containing the pDHFR7 plasmid gave reduction of folate ($4 \times$ background). Additional evidence that the reductase encoded by the pDHFR7 plasmid is of mammalian origin was obtained by inhibitor analysis (Fig. 5). The DHFR isolated directly from mouse cells and the activity encoded by the pDHFR7 plasmid showed identical sensitivities to methotrexate, trimethoprim and a triazine derivative (2,4,-diamino-1-(4'-butylphenol)-6,6 dimethyl-1, 6-dihydro-1,3,5-triazine); both enzyme activities were 200 times more sensitive to the triazine than to trimethoprim. In contrast, bacterial dihydrofolate reductase is inhibited more effectively by trimethoprim ($K_i = 5 \times 10^{-9}$) than it is by triazine ($K_i = 6.5 \times 10^{-4}$)²⁵.

As methotrexate binds stoichiometrically to dihydrofolate reductase²⁷, we can estimate the number of molecules of enzyme in the pDHFR7 plasmid extracts from the methotrexate inhibition data of Fig. 5. We calculate from the specific activity of the extract (3 units per mg of soluble protein) and the specific activity and methotrexate binding parameters of the mouse enzyme²⁸ that 0.01% of the soluble bacterial protein is active mammalian DHFR.

Immunological characterisation of bacterial cell extracts containing mouse DHFR

Immunological evidence confirming the nature of the DHFR encoded by pDHFR7 and other plasmids that contain a mouse DHFR cDNA insert was obtained using a solid-phase sandwich radioimmunoassay¹². Tp^R clones of χ 2282 containing the independently derived plasmids pDHFR 7, 12 and 13 showed a strong reaction with rabbit antibody directed against mouse DHFR in an *in situ* immunoassay¹² (data not shown); protein that reacted with the antibody was also made by bacteria which showed low levels of phenotypic expression and by some clones that did not make a biologically functional DHFR (that is, were Tp sensitive). The nature of the antigen synthesised by Tp^S and Tp^R clones was examined more fully using a newly developed method (filter affinity transfer, or FAT procedure) for the *in situ* immunological characterisation of proteins in gels²⁹. This procedure depends on the covalent coupling of F(ab)₂ antibody fragments to a chemically derivatised and activated cellulose filter; antigen transferred on to the filter from an SDS-polyacrylamide gel is detected by subsequent incubations with antiserum and ¹²⁵I-labelled *Staphylococcus aureus* protein A (ref. 12).

Filter affinity transfer analysis of the pDHFR7 extract (Fig. 6, lanes b, c) shows the presence of protein that reacts immunologically with the antibody to mouse DHFR and further shows

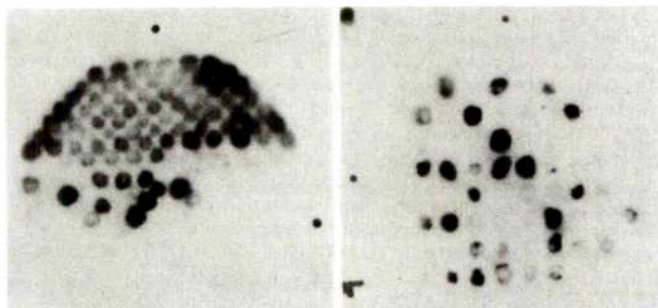


Fig. 3 Detection of colonies containing DHFR cDNA inserts by *in situ* hybridisation. Colonies were screened for DHFR sequences using a modification (G. N. Duell, unpublished) of an *in situ* hybridisation procedure¹¹. Tc-resistant colonies were transferred to nitrocellulose filters (Millipore, HAWG) that had been placed on Penassay broth agar plates containing Tc (10 μ g per ml). (Filters had been washed twice by boiling in H₂O and autoclaved before being placed on plates.) After 2–3 d of bacterial growth at 32°C, the filter was removed from the plate and placed on a Whatman no. 3 pad saturated with 0.5 M NaOH. After 7 min, the filter was sequentially transferred to a series of similar pads saturated with 1 M Tris, pH 7.4 (twice, 7 min each); 1.5 M NaCl, 0.5 M Tris, pH 7.5 (once, 7 min); and 0.30 M NaCl, 0.03 M Na citrate (2 $\times$ SSC) (once, 7 min). After the excess liquid had been removed by suction, the filter was placed on a pad containing 90% ethanol, dried by suction and baked *in vacuo* at 80°C for 2 h. Before hybridisation, filters were pretreated for 3–6 h at 65°C in hybridisation buffer that contained 5 $\times$ SSC, pH 6.1 0.2% SDS, 0.02% Ficoll 400 (Pharmacia) and 8 μ g ml⁻¹ *E. coli* tRNA. Hybridisations were carried out with individual filters in 1.5 ml hybridisation buffer containing 2 $\times$ 10⁴ c.p.m. ³²P-labelled purified DHFR cDNA¹³ in a sealed plastic bag at 65°C for 24 h. The filters were then washed in hybridisation buffer (once, 60 min at 65°C); in 5 $\times$ SSC, pH 6.1 (three times, 60 min each at 65°C); and in 2 $\times$ SSC, pH 7.4 (twice, 10 min at room temperature), air dried, and prepared for autoradiography. Left: top, a collection of χ 1776 colonies which contain a DHFR cDNA insert; middle, Tc-resistant χ 2282 colonies derived from transformation with annealed pBR322 ds cDNA—both reacting and non-reacting colonies are seen; bottom, colonies containing pBR322 and pACYC101 plasmids which show no visible hybridisation. Right: several positive colonies on a representative filter analysed in screening χ 1176 transformants. Negative colonies represent clones containing pBR322 or pACYC101 plasmids which show no visible hybridisation.



Fig. 4 Map of cDNA insert and adjacent regions of pDHFR 7 plasmid. Endonuclease cleavage map and partial DNA sequence of cDNA insert of the pDHFR 7 plasmid. The shaded area indicates the structural sequence for mouse DHFR. The locations of endonuclease cleavage sites were determined from polyacrylamide gel electrophoresis patterns following simultaneous or sequential digestions of either intact pDHFR or fragments isolated after earlier digestions. Restriction endonucleases (New England Biolabs or Bethesda Research Laboratories) were used according to the vendor's recommendations. Cleavage sites listed above the shaded area were assigned to specific amino acid positions within the mouse enzyme by nucleotide sequence. The boxed numbers of the *HpaII* and *PstI* sites indicate the locations of the sites in the pBR322 plasmid as determined by Sutchiff (personal communication), and were used in orientating the amino acid sequence of the DHFR gene with respect to the β -lactamase sequence. The nucleotide sequence in the vicinity of the pBR322-cDNA junction corresponding to the 5' end of the mRNA used as template for the cDNA, and the sequence in the region of the *HpaII* site at amino acid 104 of the structural sequence are shown. Nucleotides and amino acids are numbered from the start of the DHFR coding sequence; nucleotides in the 5' direction on the mRNA from position 1 have negative numbers. The DNA sequence shown were determined by the method of Maxam and Gilbert⁴⁰. Fragments (*d* + *c*) and (*a* + *b*) were 5' end-labelled at the *HpaII* and *BglII*-generated ends, treated with *HaeIII* endonuclease, and subjected to electrophoresis in 6% acrylamide gel and TBE buffer³⁹. Fragments *a*, *b* and *c* were eluted from the gel³², precipitated with ethanol and used directly for base sequence determination using the gel system described in refs 40 and 41.

that most of the immunologically reactive material has the same electrophoretic mobility as enzyme obtained directly from mouse cells (molecular weight 22,000) (Fig. 6, lane *a*). An immunologically reactive band that migrates at this position was also seen in the material eluted with folic acid from a methotrexate affinity column that contained an extract from χ 2282 (pDHFR7) cells (Fig. 6, lane *d*), suggesting that the 22,000 MW protein made by these bacterial cells has binding sites for both methotrexate and folate. Additional immunologically reactive bands which have mobilities consistent with a MW of 30,000–90,000 were seen in varying amounts in different extracts of bacterial cells that contained pDHFR7 (Fig. 6, lanes *b*, *c*); as the 1,500-base pair insert in this plasmid is capable of coding for a polypeptide no larger than 50,000 MW, we conclude that the most slowly moving bands are likely to be hybrid proteins that include antigenic sites of the mouse DHFR. Immunologically reactive high MW proteins are also made by a *Trp*⁸ clone (pDHFR21, Fig. 6, lane *e*) which contains a DHFR cDNA insert; however, this clone fails to synthesise an immunologically reactive 22,000 MW band. No immunological reactivity with

antibody made to mouse DHFR was detected in extracts of cells carrying only the pBR322 vector (Fig. 6, lane *f*).

Variation in level of expression of mouse DHFR cDNA sequences in bacteria

Thirty-two separately derived clones of χ 2282 transformants that had been selected on plates containing only Tc were replated on medium containing both Tc and Tp, and also were tested for the presence of a DHFR cDNA insert by *in situ* hybridisation. Fourteen colonies contained sequences homologous with the purified DHFR cDNA probe, and five of these (termed 'weak expressors') grew on plates containing 5 μ g ml⁻¹ or more Tp. The remaining nine clones that contained DHFR sequences failed to show any growth on concentrations of Tp above 2.5 μ g ml⁻¹, and were termed 'non-expressors'. Plasmid DNA isolated from four of the nine non-expressors and from all five weak expressors was analysed by gel electrophoresis, and the mean inhibitory concentration (MIC) of Tp was determined for each of the clones. The structural relationship of the cDNA

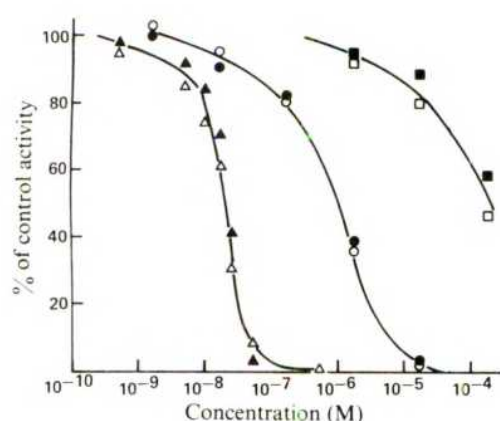


Fig. 5 Inhibitor analysis of DHFR from bacterial cells. Stationary phase cultures of χ 2282 expressing trimethoprim resistance were grown in the presence of Tp ($1 \mu\text{g ml}^{-1}$) in minimal medium, washed with isotonic saline, and suspended in 50 mM potassium phosphate buffer, pH 7.0, containing 10 mM benzamidine and 10 mM phenyl methyl sulphonyl fluoride (3 volumes buffer to 1 volume cells). The suspension was sonicated and centrifuged at 10,000 r.p.m. for 15 min. The supernatant was centrifuged for 1 h at 100,000g before being studied. An R_2 methotrexate-resistant mouse cell extract was prepared as described elsewhere⁴². Enzyme activity was measured by the radioactive folic acid assay previously described²⁸. Protein was determined by the method of Lowry⁴³. Approximately 3 units of activity from the χ 2282 extract or 5 units from the methotrexate-resistant mouse cell extract were incubated with inhibitor for 10 min at 24°C before assaying for folate reductase activity; the concentrations shown represent the final concentration of inhibitor in the reaction mixture. Background values, determined by measuring enzyme activity in the presence of 10 mM methotrexate, have been subtracted from all points. The results presented are the average of duplicate samples which generally varied by less than 10% and are expressed as a percentage of the value obtained in the absence of inhibitor. One unit of activity is the amount of enzyme needed to reduce 1 nmol of folate in 15 min at 37°C. Δ and $\blacktriangle$ indicate addition of methotrexate, $\circ$ and $\bullet$ indicate addition of the triazine derivative, and $\square$ and $\blacksquare$ indicate trimethoprim addition for χ 2282 and the mouse cell extracts, respectively.

insert to the β -lactamase gene sequence of the vector, the length of each insert, and the minimal inhibitory concentration (MIC) determined for the clone are shown in Fig. 7. As can be seen, plasmids pDHFR7, 12, 13 and 26–29 all contain a complete DHFR structural sequence inserted in the same orientation (that is, orientation a) as the gene encoding the bacterial β -lactamase. The clone carrying each of these plasmids expresses Tp resistance, although the MIC varies from $150 \mu\text{g ml}^{-1}$ for pDHFR28 to $>1,000 \mu\text{g ml}^{-1}$ for pDHFR7, 12 and 13; the greatest reactivity with antibody to mouse DHFR occurs with pDHFR12 (unpublished data).

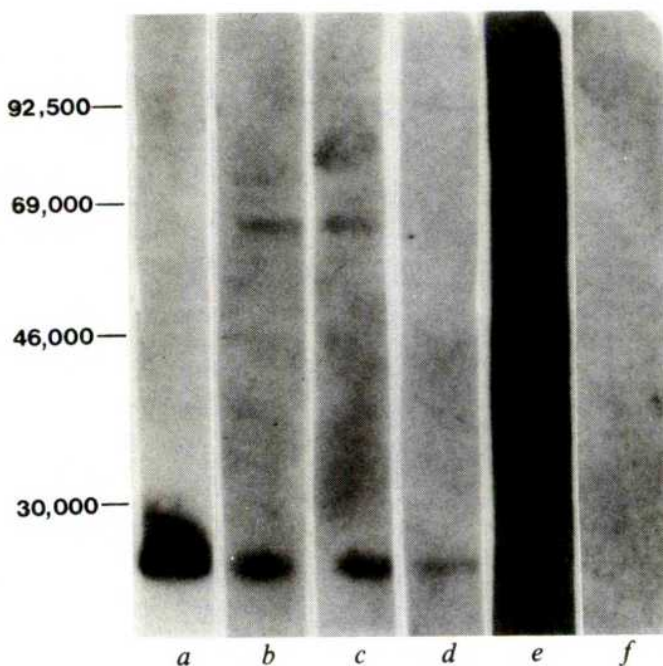
It is unlikely that translational reading frame is the determining factor in the different levels of expression observed in these clones, as our DNA sequence analysis indicates that a correct reading frame is not essential for efficient expression in pDHFR 7, 12 or 13. However, the positioning of the putative ribosomal binding site in relation to the ATG start codon may potentially influence the strength of expression by affecting the formation of the translational initiation complex (compare with ref. 23) (Figs 4, 7).

The end of DHFR structural sequence that corresponds to the 5' end of the mRNA is not present in plasmids pDHFR 21 and 24, thus explaining the observed lack of functional expression of the cDNA in these clones. However, pDHFR21, which has lost less than 15% of the structural sequence, nevertheless encodes a (probably hybrid) peptide that contains antigenic sites which

react with antibody to mouse DHFR (Fig. 6 and unpublished data). It is particularly interesting that the clone carrying pDHFR25 expresses a low level of Tp resistance, although the coding sequence for the DHFR enzyme is inserted in an orientation opposite to that of the β -lactamase gene. This finding, and our detection in extracts of the pDHFR25 clone of protein that reacts immunologically with antibody to the mouse enzyme (unpublished data), suggest that readthrough transcription into the DHFR coding sequence from a promoter sequence located on or near the distal segment of the β -lactamase gene may occur. Consistent with this interpretation are preliminary data suggesting that DHFR antigenic sites are also synthesised by cells carrying pDHFR23, which is a non-expressor of Tp resistance and contains a cDNA insert in orientation b (Fig. 7). Further study is required to determine whether sequences in the distal segment of the β -lactamase gene are capable of serving as weak promoters for the initiation of mRNA chains that extend into the DHFR cDNA.

The findings reported here indicate that the bacterial clones we have constructed are synthesising and phenotypically expressing DHFR encoded by mouse cDNA sequences: (1) the cDNA insert cloned in bacteria has been shown by *in situ* hybridisation to be homologous with the mouse gene and by direct DNA sequence analysis to encode the amino acid sequence of mouse DHFR, (2) DHFR enzymatic activity and resistance to Tp are specified by nucleotide sequences present on chimaeric plasmids but not on the vector, (3) the DHFR

Fig. 6 Filter affinity transfer analysis²⁹ of bacterial cell extracts. 20 μl of extracts in SDS sample buffer were run at constant current for 3 h in an 11.25% SDS-polyacrylamide slab gel. The gel was incubated in PBS (50 mM phosphate buffer containing 0.15 M NaCl) for 30 min and placed on a blotter wet with PBS. Peptides were specifically transferred from the gel to strips of a dry cellulose filter that had been covalently coupled to anti-DHFR F(ab)₂ fragments¹². Filters were washed, incubated with antibody to DHFR, washed again and treated with ¹²⁵I-labelled protein A. After additional washing and drying steps, the filters were analysed by autoradiography. The eluate fraction (lane d) was obtained by passage of an extract of χ 2282 cells containing pDHFR7 over a 0.5 ml methotrexate-Sepharose affinity column. The extract was acidified to pH 5.8, passed over the column and the bound fraction was eluted with 2 mM folic acid in a 5 mM NaHCO₃ buffer at pH 8.5 as described elsewhere²⁸. Lane a, extracts of mouse cell line; lanes b and c, pDHFR 7; lane d, eluate from methotrexate column; lane e, pDHFR21; lane f, pBR322.



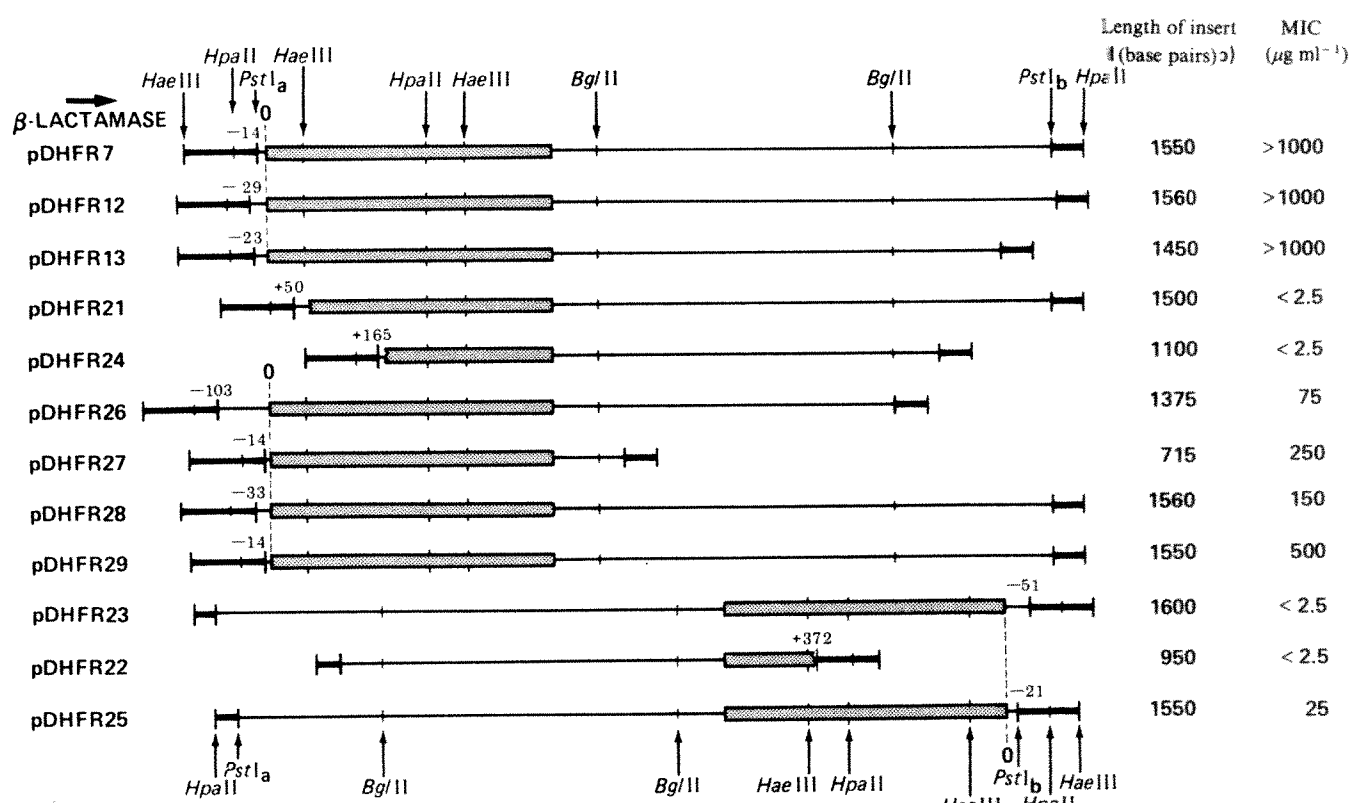


Fig. 7. Some structural and functional properties of chimaeric plasmids and χ 2282 clones containing DHFR cDNA insert. The MIC of Tp for each of the χ clones was tested on M9 minimal agar plates containing biotin ($2 \mu\text{g ml}^{-1}$), casamino acids (0.5%), DA_2 ($50 \mu\text{g ml}^{-1}$) and Tp concentrations ranging from 0 to $1,000 \mu\text{g ml}^{-1}$. χ 2282 (pBR322) was used as a control and is sensitive to Tp at $2.5 \mu\text{g ml}^{-1}$, as determined by incubation at 32°C for 3 d. Plasmid DNA isolated from each clone was digested with *Pst*I endonuclease¹⁵ at 37°C for 3 h and extracted sequentially with phenol and ether. DNA was precipitated with ethanol, resuspended in $8 \mu\text{l}$ TE buffer and electrophoresed in 1.2% agarose gels in TBE buffer to determine the length of the inserted fragment. ColEI plasmid DNA digested with *Hae*II endonuclease⁴² was added to each sample as an internal molecular weight standard. Additionally, *Hind*III-generated fragments of SV40 DNA were used as an external standard⁴⁵. The standard error is ± 100 base pairs with the method used. The orientation of the cDNA inserts in the vector plasmid was determined by gel analysis of plasmid DNA digested with *Bgl*II and *Hinc*II endonucleases; in addition, pDHFR25 was treated with the *Eco*RI and *Bgl*II enzymes to confirm the orientation of its insert. The direction of transcription of the β -lactamase gene of pBR322 is indicated by an arrow. The shaded area in each plasmid map indicates the structural sequence for DHFR. The numbers shown above each *Pst*I site indicate the distance (in base pairs) between the cleavage point and the first nucleotide of the DHFR structural sequence as described in Fig. 4. This distance was determined exactly by DNA sequence analysis for plasmids pDHFR 7, 12 and 13 and was estimated for the other plasmids by gel electrophoresis of fragments produced by either the *Hae*III or *Hpa*II endonucleases. For the chimaeric plasmids that we subsequently sequenced, this estimate proved to be accurate within 5 base pairs. *Pst*I–*Bgl*II distances were determined for the chimaeric plasmids by gel electrophoresis using *Hpa*II-cleaved pBR322 DNA as an internal standard. Nucleotides in the 5' direction on the mRNA from position 1 have negative numbers and are estimates obtained from gel analysis for all plasmids except pDHFR7 and 12.

encoded by the constructed plasmids shows differential sensitivity to competitive inhibitors of DHFR characteristic of the mammalian gene product, and (4) the enzyme synthesised by bacteria containing DHFR cDNA is immunologically reactive with antibody made against mouse DHFR.

Note that the clones which express the highest levels of Tc resistance contain an immunologically reactive peptide having a size characteristic of the mammalian DHFR. A peptide of this size could potentially result from proteolytic cleavage of a fused β -lactamase–DHFR protein that was initiated at the β -lactamase ribosomal binding site. This proposal is consistent with the finding of large-sized protein species containing DHFR antigenic sites in extracts from cells that are either Tp^R or Tp^S (Fig. 6). More intriguing, however, is the possibility noted above that the *Pst*I–poly dG sequence constructed at the vector–cDNA junction can act together with the nearby ATG (AUG) translational start codon to bind mRNA to the bacterial ribosome and initiate DHFR peptide chains within the cDNA insert. If this interpretation is correct, initiation of peptide chains within other eukaryotic cDNA inserts may be obtainable in bacteria by use of the same structural relationships that have resulted in expression of the mouse DHFR coding sequence. Additional DNA

sequence analysis and investigation of the protein products encoded by chimaeric plasmids should provide definitive information on this point and should help elucidate further the structural basis for the different levels of Tp resistance expressed by various clones.

Some of the bacterial clones we have isolated produce proteins that react immunologically with antibody to mouse DHFR but which are not biologically active. Our results suggest that an important obstacle to functional expression of mammalian DNA sequences in bacteria has been the development of an assay capable of detecting those clones that possess both a complete coding sequence and the correct nucleotide relationships to allow such expression. The strong phenotypic selection possible in the present experiments has provided an effective means of identifying and isolating expressing clones.

As the cloned coding sequence for mouse DHFR is selectable in higher organisms as well as in bacteria, it constitutes a powerful tool for the construction of eukaryotic cloning vectors, for the isolation of replication regions of eukaryotic chromosomal and extrachromosomal genomes (compare ref. 30), and for the isolation and characterisation of signals that control genetic transcription and translation in variety of species.

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letters to nature

N Galaxies—a new class of X-ray sources

BURBIDGE¹, classified extragalactic objects with bright nuclei into three classes: in order of increasing optical luminosity these are (1) Seyfert galaxies; (2) N galaxies; (3) quasars. Many Seyferts and several quasars have been shown to be X-ray sources. Here we show that N galaxies are also powerful X-ray sources. In fact, all six N galaxies in the 3C radio catalogue with redshifts less than 0.06 (ref. 2) are detected by the first full-sky survey of the Goddard Space Flight Center detectors (A2) on HEAO 1. X-ray emission has also been discovered from a strong Southern Hemisphere radio source, the N galaxy Pic A. Six of the seven objects are classified as broad-line radio galaxies (BLRGs)^{3–5} and one (3C371) is classified as a BL Lac object⁶. These objects are differentiated from the newly discovered class of 'emission line' galaxies⁷ by their high radio flux and extremely broad lines. Before HEAO 1 there was one confirmed N galaxy, 3C120 (ref. 8), and one suggested, 3C390.3 (refs 9, 10). We detect 3C120, strengthen the identification of 3C390.3, and present evidence for X-ray emission from four new sources, 3C111, 3C382, 3C371, and Pic A. We also suggest 3C445 as the identification of 2A2220-022 (ref. 11).

Figure 1 shows 90% confidence error boxes on the positions of these seven galaxies together with 4U (ref. 10) or 2A (ref. 11) error boxes where applicable. The 2–10 keV luminosity of these objects, as well as other relevant information, is given in Table 1. We have also looked for X-ray flux from the next closest N galaxies, PKS0521–36 and 3C227. No significant flux was seen, but the 90% upper limit on the X-ray luminosities (given in Table 2) are consistent with their luminosities being comparable to the seven detected N galaxies. Except for quasars, these N

galaxies are the most luminous class of compact X-ray sources yet detected.

Grandi and Osterbrock have classified radio galaxies spectroscopically as BLRG or narrow-line radio galaxies (NLRG) and have demonstrated a high degree of association between the classification 'broad-line' and the morphological type 'N'. In marked contrast to the situation for BLRGs, only 3C405 (Cyg A) of the five NLRGs listed by Grandi and Osterbrock, with redshifts less than 0.06, is a detected X-ray source. 3C317 is confused with Ab2052, a distance class 3 cluster, and so its luminosity is poorly determined. Upper limits on the flux from the other three NLRGs are given in Table 2. The deduced upper limits on the luminosities are considerably less than average X-ray luminosities for BLRG of $\sim 3 \times 10^{44}$ erg s⁻¹. Note that of the detected N galaxies, the one with the narrowest emission lines, 3C371, has the smallest X-ray luminosity.

The lack of X-ray emission from NLRGs contrasted to the virtual certainty of X-ray emission from BLRGs is similar to the distinction between type 1 and type 2 Seyferts, that is, none of the ~20 known X-ray emitting Seyfert galaxies are classical type 2 Seyferts. Grandi and Osterbrock have drawn attention to the optical spectral similarities and differences between BLRGs and type 1 Seyferts, and NLRGs and type 2 Seyferts, respectively.

The fact that many of these N galaxies possess compact radio components suggests that the synchrotron self-Compton process (SSC)¹² may be the principle cause of the X-ray emission. For 3C390.3, the best studied of these objects, a SSC model predicts a physical size to the X-ray emitting region of ~0.1 pc and a time scale for X-ray variability of months¹³. The detection of 3C390.3 by Uhuru and Copernicus at considerably higher flux levels and an Ariel 5 upper limit on 3C382 (ref. 14) ~20% lower than the present detection indicate possible variability in

Table 1 Positive detection of N galaxies

Object	X-ray name	Z	L_x HEAO* (2–10 keV)	Previous L_x	Notes
3C111	H0415+37	0.0485	5.6×10^{44}	—	Near 3U0430+37
3C120	2S0430+05	0.033	1.4×10^{44}	2.3×10^{44} (ref. 7)	SAS-III position
3C371	H1807+69	0.0506	0.5×10^{44}	—	BL Lac object
3C382	H1832+32	0.0586	5.5×10^{44}	$<4.5 \times 10^{44}$ (ref. 14)	—
3C390.3	4U1847+78	0.0569	2.4×10^{44}	4.4×10^{44} (ref. 9) 12×10^{44} (ref. 10)	Variable ⁹
3C445	2A2202-02	0.0568	4.5×10^{44}	7×10^{44} (ref. 11)	Variable?
Pic A	H0519-45	0.0342	0.8×10^{44}	—	—

* Luminosity converted assuming all spectra are like that of 3C390.3 and $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$. Statistical errors are $\sim 20\%$ for weakest source.

this class of object. However, results from the second complete HEAO 1 sky scan will provide a better test of variability in these objects because of the difficulty in comparing the fluxes of these weak sources between different satellite experiments. Preliminary analysis of data from the first HEAO 1 survey indicates no variability ($\pm 50\%$) for these sources on a day-to-day basis.

Alternatively, Fabian¹⁵ has suggested that the X rays are produced by gas clouds colliding with velocities of the order of the Doppler width of the broad lines. This would account for the strong selection seen between BLRGs and NLRGs and the hard spectrum seen in these objects. (Detailed spectral analyses are in progress and will be reported elsewhere.) Only simultaneous radio and X-ray observations similar to that already performed for Cen-A¹⁶ can distinguish between these two mechanisms.

Further HEAO 1 observations will provide the opportunity to determine the detailed spectra of these objects and their variability on a time scale of months. Also, further BLRGs, when they are discovered optically, may be identified with some of the ~ 20 new¹⁷, weak, unidentified sources discovered with the HEAO 1 A2 experiment.

Table 2 90% Confidence upper limits

Name	Z	L_x (90%)	Notes
3C98	0.0306	$<0.5 \times 10^{44}$	NLRG
3C178	0.0073	$<0.02 \times 10^{44}$	NLRG
3C192	0.0596	$<1.0 \times 10^{44}$	NLRG
3C227	0.0861	$<3.3 \times 10^{44}$	BLRG
3C317	0.0351	$<1.6 \times 10^{44}$	NLRG confused with distance class 3 Abell cluster 2052. New source H1513+07
PKS0521-36	0.061	$<1.5 \times 10^{44}$	N galaxy

We thank A. Fabian for suggesting 3C382 as an X-ray source and S. Pravdo and J. Swank for their help. F.E.M. and R.F.M. are NAS/NRC research associates. The A2 experiment on HEAO 1 is a collaborative effort led by E. Boldt of GSFC and G. Garmire of CIT with collaborators at GSFC, CIT, JPL and UCB.

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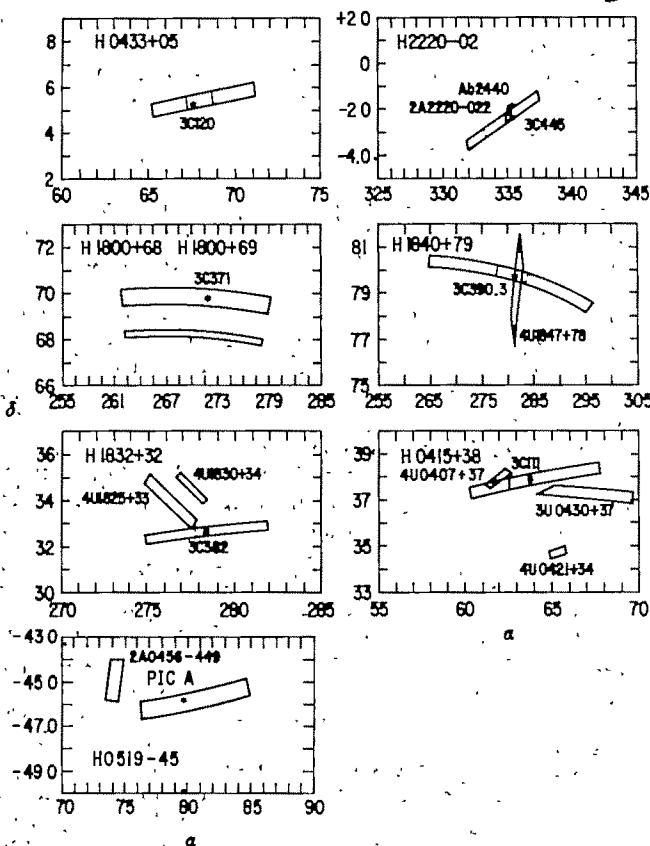


Fig. 1 HEAO 1 A-2 90% confidence error boxes. Other error boxes indicated where applicable. The asterisks indicate the candidate object. The dashed lines indicate 90% confidence error boxes under the assumption that the source intensity did not vary during the observation. In the case of 3C371 there is an additional new source H1800+69 indicated. On H220-02 the smaller box is 2A220-022.

The solar wind
at the turn of the century

GEOMAGNETIC ACTIVITY is driven by the solar wind, so information on the past history of the wind can be derived from studies of geomagnetic indices¹⁻³. Comparison of geomagnetic activity and sunspot number over the past hundred years shows that although both vary with the 11-yr solar cycle, they do so with characteristically different patterns. Geomagnetic activity rises and falls with about the same amplitude over each 11-yr solar cycle, but these variations seem to be superposed on a longer-term cycle of similar amplitude. Consequently there is a long-term variation in the 11-yr cycle minima as well as maxima. In contrast, in the sunspot number record the long cycle appears as changing amplitudes of the 11-yr cycles, with sunspot number returning to near zero at each 11-yr minimum. As geomagnetic activity reflects solar wind conditions, it is possible to extrapolate backwards in time the present-day solar wind measurements taken near the long-cycle maximum and we estimate here that around 1900, near the long-cycle minimum, either the average solar wind speed was as much as a factor of two lower, or the average southward component of the solar wind field was as much as a factor of three smaller, or both parameters were significantly less than at present. Thus, in the not too distant past the solar energy output by means of the solar wind was different from what it is now.

That the solar cycle variations of sunspot number and geomagnetic or auroral activity are different has been known for some time. Fritz⁴ first noted that the auroral cycle lags the sunspot cycle. More recently, spectral analyses of the magnetic activity index A_p and sunspot number show that both series have the same strong line at 10.2 yr and that the lag noted by Fritz is of 18 months duration⁵. The shapes of the two solar-cycle variations are also different. Magnetic activity tends to have a secondary, post-sunspot-maximum peak which is associated with recurrent storms⁶.

The difference between geomagnetic activity and sunspot number that we discuss here concerns the pattern of the minima from cycle to cycle. Figure 1 shows the solar-cycle variations of yearly averages of sunspot number and aa index of geomagnetic activity in the top and middle panels, respectively, for those cycles from the 1900s to the 1960s where the $\langle aa \rangle$ minima

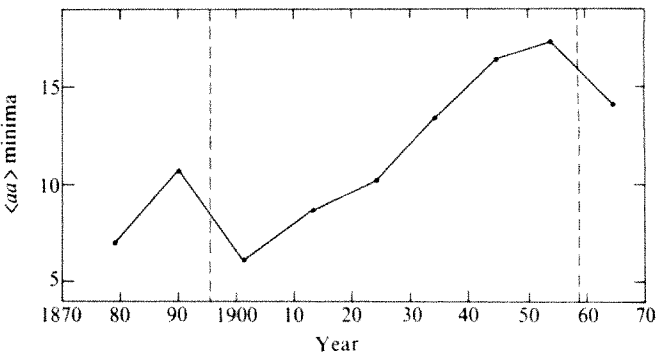


Fig. 2 Solar cycle minima of yearly means of aa . Points between dashed lines increase almost linearly.

increase almost linearly in time. The total increase is about 10 units, an amount typical of the change in $\langle aa \rangle$ over a single solar cycle. The slope of the increase is so steep that $\langle aa \rangle$ at the 1910 maximum is about the same as $\langle aa \rangle$ at the 1954 minimum. In striking contrast is the behaviour of the average sunspot variation. Over the same period the maxima increase by about 120 units; but at each solar minimum the sunspot number returns to near zero.

The increase in sunspot maxima is part of the well-known '80-yr' sunspot cycle, and apparently the rise in the $\langle aa \rangle$ minima is the geomagnetic activity or solar wind manifestation of this same cycle. Brown⁷ has recently shown that although the sunspot number falls to very low values at minima, the 80-yr cycle is also clearly present in the time variation of the minima. In addition, he found that the phase difference between the variations of the minima and the maxima is such that the level of any given minimum is a good predictor of the level of the subsequent maximum.

Figure 2 shows the minima of $\langle aa \rangle$ as a function of time. A root mean least squares fit to the six points between 1900 and 1960 is given by

$$\langle aa \rangle_{\min} = 0.22 (T - 1900) + 5.70 \tag{1}$$

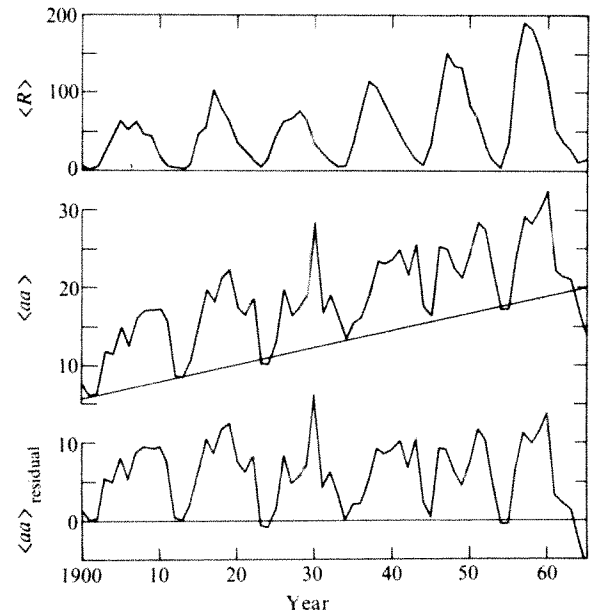
with a correlation coefficient of 0.99. The variation of these six points is remarkably linear. The possibility that the linear increase is caused by a systematic error in $\langle aa \rangle$ owing to some change occurring at one of the two stations used to determine the daily aa can quickly be ruled out because almost identical increases occur at the two antipodal stations (Melbourne and Greenwich) used to derive the index. The linear increase is apparently the result of a change in the driver of geomagnetic activity—the solar wind.

Because in the case of geomagnetic activity the 11-yr cycle appears to be superposed on or added to the long-term cycle, the pattern of the 11-yr cycle can be separated from the long-term cycle by defining a quantity $\langle aa \rangle_{\text{residual}}$ given by

$$\langle aa \rangle_{\text{residual}} = \langle aa \rangle_{\text{measured}} - \langle aa \rangle_{\min} \tag{2}$$

where $\langle aa \rangle_{\min}$ is defined by equation (1). The values of $\langle aa \rangle_{\text{residual}}$ are shown in the lower panel of Fig. 1. The important point to note is that the amplitudes of the cyclic changes are all about the same. The residual $\langle aa \rangle$ averaged over each cycle varies only from a high of 7.2 for the 1910s to a low of 5.8 for the cycle beginning in 1924. The shapes of the solar cycle variations of residual $\langle aa \rangle$ are almost square. Post-sunspot-maximum activity peaks are clearly present but less pronounced relative to the first peak in each cycle as compared to the solar cycle variations of $\langle aa \rangle$ in the middle panel. The first peak does not necessarily occur at sunspot maximum, but, on the average, precedes maximum. In the two solar cycles preceding those shown in Fig. 1, the first peaks are well pronounced and also occur before sunspot maximum (see Fig. 4 of Mayaud⁸). Note that these findings are not consistent with the usual association of first peaks with flare-induced geomagnetic storms, which are expected to occur most frequently at sunspot maximum.

Fig. 1 Yearly means of sunspot number, the aa index, and residual aa during the rising years of the long cycle.



Following the suggestion of Hirshberg⁹ that the 11-yr geomagnetic activity cycle, which is so different from the sunspot cycle, may well represent an 11-yr cycle of the solar wind, N.U.C. *et al.*¹⁰ found a remarkable similarity between the variations of geomagnetic activity and solar wind speed over the past solar cycle. To provide good correlations on both short and long time scales, they derive an empirical relationship between geomagnetic activity and the product of the square of the solar wind speed V and the southward component of the interplanetary magnetic field B_s . With a relationship of this kind we can estimate what the solar wind speed and/or magnetic field intensity may have been in 1901.

The regression line of $\langle aa \rangle$ on $\langle B_s \rangle \langle V^2 \rangle$ for data from the past solar cycle is

$$\langle aa \rangle = 1.3 + 4.7 \times 10^{-5} \langle B_s \rangle \langle V^2 \rangle \quad (3)$$

where B_s is measured in units of nT and V in km s^{-1} . The correlation coefficient is 0.9. Equation (3) was used to draw the curves of constant $\langle B_s \rangle \langle V^2 \rangle$ in Fig. 3 for the maximum and

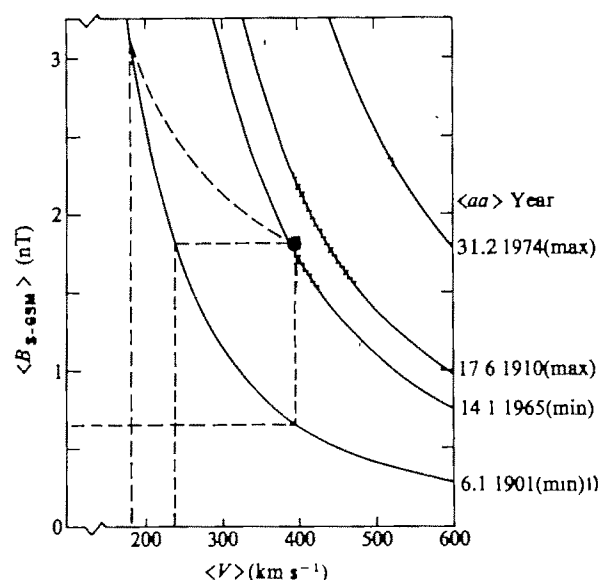


Fig. 3 Curves of constant $\langle B_s \rangle \langle V^2 \rangle$ for the values of $\langle aa \rangle$ listed in the right margin. Shaded area indicates ranges of yearly averages of observed values. Dashed lines extrapolate variables from the 1965 minimum to the 1901 minimum.

minimum $\langle aa \rangle$ for solar cycle 14 beginning in 1901, and for cycle 20 beginning in 1965. Cycle 20 occurs just after the peak in the long cycle, and cycle 14 occurs at the long-cycle minimum. Thus, the overlap in the areas covered by these two cycles in Fig. 3 is small. Cycle 19, with an $\langle aa \rangle$ maximum of 32.9 and minimum of 17.3, would give essentially no overlap; but cycle 20 is plotted in Fig. 3 because it begins the era of spacecraft measurements. The ranges of observed $\langle B_s \rangle$ and $\langle V \rangle$ are indicated by the shaded rectangle, and the encircled datum point marks the averages $\langle B_s \rangle = 1.82$ nT and $\langle V \rangle = 395$ km s^{-1} for the $\langle aa \rangle$ minimum year 1965. Note that the observed $\langle B_s \rangle$ for 1965 is not the lowest observed value, as indicated by the shaded region, which extends down to $\langle B_s \rangle = 1.53$ nT. This lowest value is an average from 1964, the year of sunspot minimum; its uncertainty is rather large, since only about one month of hourly averages are available from 1964.¹¹ Interestingly, if curves were drawn for the five $\langle aa \rangle$ minima recorded prior to 1933, none of them would intersect the shaded region of observed solar wind values.

If in one extreme we assume that the solar wind speed in 1901 was the same as during $\langle aa \rangle$ minimum of the recent cycle, it follows that the magnitude of $\langle B_s \rangle$ was about 0.65 nT, as indicated in Fig. 3 by the intersection of the dashed line at $\langle V \rangle = 395$ km s^{-1} and the 1901 curve. This value of $\langle B_s \rangle$ is about a factor of three smaller than $\langle B_s \rangle$ for the 1965 $\langle aa \rangle$ minimum.

As mentioned by Russell², a reduced southward component can result either from a reduction in the intensity of fluctuations relative to the field magnitude, since an undisturbed solar wind has no significant southward component in the solar equatorial plane, or, if that ratio is constant, from a reduction in the field magnitude. Thus if the ratio of the southward component to the field magnitude was the same in 1901 as at present, then the field magnitude may have decreased by as much as a factor of three.

In the other extreme, we can assume that the solar wind field remained constant, and the speed decreased to produce the 1901 $\langle aa \rangle$ minimum. If $\langle B_s \rangle$ remained at the 1965 value of 1.82 nT, then the dashed line at that value in Fig. 3 indicates that the solar wind speed average was 237 km s^{-1} in 1901. A more physically correct treatment of this extreme is to assume that the field magnitude at the Sun and the ratio of $\langle B_s \rangle$ to the field magnitude at 1 AU remained constant. In this case, the increase in field magnitude with decreasing speed at 1 AU must be taken into account. The resulting dashed curve in Fig. 3, which was plotted from Parker's¹² equations, indicates that under these assumptions $\langle V \rangle$ was as low as 180 km s^{-1} . This is indeed a low value for a yearly average in comparison to solar wind measurements of recent times, where hourly averages of ~ 200 km s^{-1} are rare. These values for speeds and field intensities are extremes, and it is more likely that both quantities changed over the 64-yr period.

Although photographs of the corona during eclipses at the turn of the century are available, the plates are not calibrated, so comparisons to the present day corona cannot be made. However, in this study we have shown that there were considerable changes in the solar wind over the past century. This implies some change in the state of the solar corona itself.

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Power harmonic radiation enhancement during the sudden commencement of a magnetic storm

THE enhancements of power harmonic radiation that occurred at the same time as a sudden commencement of a geomagnetic storm (ssc) and with geomagnetic pulsations that followed the ssc are described and discussed here. The power harmonic radiation was detected by an ELF-VLF wave receiver with two loop antennas and a vertical whip at Riverton, while geomagnetic variations were observed by three induction

magnetometers distributed at Thompson (55.9° N, 97.4° W), Riverton (51.0° N, 97.0° W) and Star Lake (50.1° N, 95.2° W), Manitoba, Canada. The power harmonic enhancements are accounted for in terms of the geomagnetically induced telluric current that was possibly shunted through transformer neutrals and a transmission line of a power system, giving rise to a near saturation of the transformer core material. The important role which the enhanced power harmonics may have in stimulation of emissions in the magnetosphere is considered.

During a joint IMS campaign for aurora/ULF-VLF waves in Manitoba, Canada, from 10 to 24 September 1977, a magnetic storm broke out on 21 September with an ssc. A generally accepted concept of an ssc was first presented in 1933¹ which in terms of hydromagnetics is described as a rapid compression of the Earth's magnetosphere due to an abrupt increase in the solar wind kinetic pressure². This specific event is ssc* in type which is characterised by a prominent negative kick on the magnetic horizontal (N-S) component before the compressional (positive) increase. Induction magnetograms at three stations, Thompson, Riverton and Star Lake indicate that the negative kick (southwards deflection) started 24.45.00 UT simultaneously at all three stations. The negative phase lasted until 20.45.47 at Thompson, 20.45.33 at Riverton and 20.45.30 at Star Lake. Following the negative phase, the compressional increase (northwards deflection) of the horizontal component took place during the intervals 20.45.47–20.47.30 at Thompson, 20.45.33–20.46.25 at Riverton, and 20.45.30–20.46.17 at Star Lake as can be seen in the lower panel of Fig. 1. This positive phase was followed by damped oscillations. At Riverton, the damped oscillation took a period of approximately 2 min and continued until 20.53.00. The ssc* and the subsequent damped oscillations in the Earth's magnetic field gave rise to two effects on VLF emissions which were recorded at Riverton concurrently with the ULF magnetic observation using a receiver system for finding the arrival direction³.

One of the two effects concerns natural VLF chorus emissions. This effect is already known and has been explained in terms of increases in gyrofrequency of suprathermal electrons and in the anisotropy factor of their distribution in velocity space during the course of a magnetospheric compression⁴. Bandwidth broadening, increase in mean frequency and enhancement of band-integrated intensity—the changes characterising the ssc effect in chorus as seen in the spectrogram in the upper panel of Fig. 1—are reasonably well understood in terms of the changes in the two parameters mentioned above.

The second effect, which is the main one considered here, is

enhancement of harmonics in commercial electric power. As the spectrogram shows, the intensities of the power harmonics increased almost concurrently with the distinctive deviations on the induction magnetograms. The main harmonic components before the ssc were the 3rd (180 Hz) and the 9th (540 Hz), both being relatively weak. At the onset of the negative kick, even harmonics came out: the bands of the 6th (360 Hz) and 12th (720 Hz) harmonics were considerably intensified. The pre-existing bands, the 3rd and 9th harmonics, seem to have been enhanced also. It is interesting that the harmonics appeared at multiples of 180 Hz as if it were the fundamental frequency. This suggests that they were generated in a three-phase a.c. transmission system. These bands then gradually decreased in intensity towards 20.45.45. They were enhanced again in a time interval 20.45.50–20.46.45 during which the harmonics up to 18th (1,080 Hz) were excited. After this, their intensity was increased again and again, for example, from 20.47.00 to 20.47.12, from 20.47.25 to 20.48.33 and so forth as shown in Fig. 1.

The initiation of the first enhancement of the harmonics (especially 6th) coincided with the initiation of the negative kick at Riverton where the VLF recording was carried out, but their decay came out about 10 s behind the positive turn of the magnetic deflection. The second enhancement then began about 15 s behind the positive turn and continued until about 20 s behind its negative turn at Riverton. The third and the fourth enhancements also occurred 15–20 s behind the corresponding deflections of the horizontal component at Riverton.

The above facts strongly suggest that the harmonics were generated in a power system near the VLF observation site at Riverton due to the effects of the ssc. One possibility is that the ssc-induced electric field gave rise to an electric current in a circuit consisting of ground-transformer A–transmission line–transformer B–ground. The current intensity must have had a slow temporal variation with a time scale comparable to that of the magnetic field variations detected by the induction magnetometers. The current probably gave rise to a quasi-d.c. bias to the hysteresis loop of the core material of the transformers, with probable magnetisation to near saturation with resultant non-linear distortion of the current waveforms⁵. Quasi-d.c. electric currents between a transformer neutral and ground have been recorded. Currents were found to increase at a time of magnetic disturbance. Enhanced currents are referred to as solar-induced-currents (SICs) in electrical engineering⁶ and were found to be as large as 100 A, during severe magnetic storms.

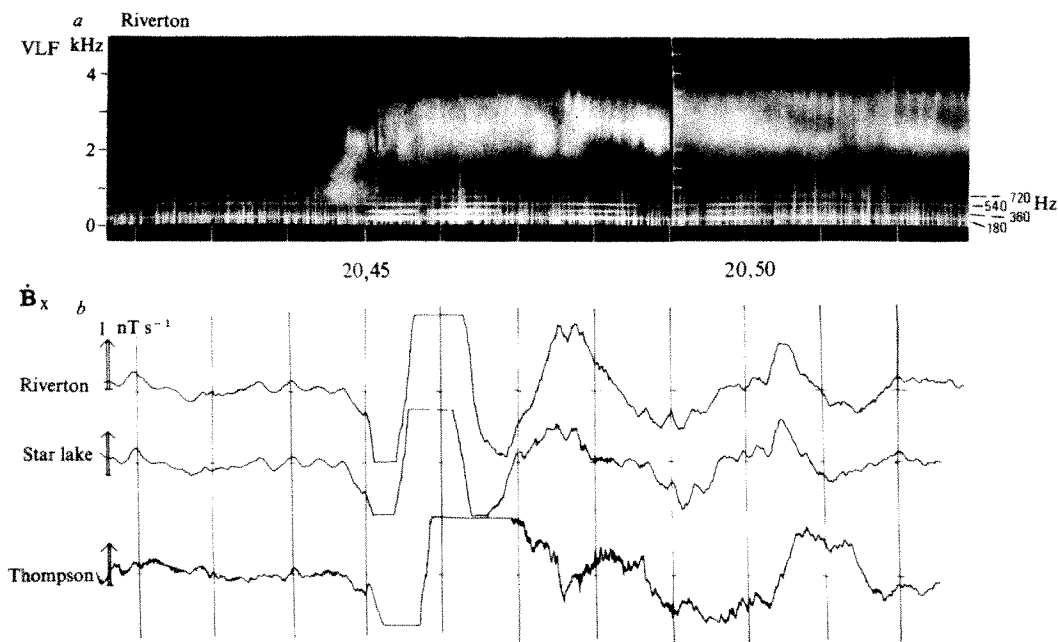


Fig. 1 A frequency-time spectrogram of chorus emission and power line harmonics (a), along with induction magnetograms of the magnetic horizontal component recorded at Riverton, Star Lake and Thompson (b). The ELF-VLF signals (magnetic component) were received on a loop antenna whose plane lay in that of the magnetic meridian. The power harmonics are seen as horizontal lines in the low frequency band below 1 kHz. Their enhancements start coincident with the initiation of the rapid negative deflection of the magnetic horizontal component.

The non-linearity in magnetisation of the transformer core materials is bound to give rise to higher harmonics in power line currents. It has been shown that the third harmonic is dominant among the higher harmonics⁷. According to our analysis, only odd harmonics are excited if the hysteresis loop of the transformer core materials has a point of symmetry, whereas both odd and even harmonics are excited in the absence of point-symmetry. In the absence of d.c. bias, the hysteresis loop of a ferromagnetic material has a point of symmetry; however, the symmetry is generally lost if a d.c. bias is applied. This readily explains why odd harmonics only (3rd and 9th) existed before the ssc whereas both odd and even harmonics (6th, 12th and 18th) were generated corresponding to the rapid magnetic fluctuations of the ssc.

The foregoing cause of SIC-induced harmonics suggests that the 10–20-s delay of the enhancements of the harmonics after the beginnings of the magnetic fluctuations on the horizontal component at Riverton may be attributed to the hysteresis of the transformer core, as the induced current must be reversed between one harmonics enhancement and the next. Possible phase differences between magnetic fluctuations and telluric currents may also be responsible for the delay.

It is appropriate to check whether the ssc-induced telluric current can be strong enough to bring the transformer cores to near saturation. If we assume that the power line is straight and l km in length, flow-pattern of the telluric currents is likely to be affected in an area of order of l^2 km². Considering a square whose side length is l km, the total current flowing across the square's side perpendicular to the power line can be estimated as follows. An abrupt magnetic field change of 1 nT is estimated to give rise to telluric currents with depth-integrated strength of ~ 1 A km⁻¹. The size of the ssc under consideration is estimated to be ~ 50 nT. Consequently, the strength of the telluric current is ~ 50 A km⁻¹. Assuming that l is 300 km, the total current flowing across the perpendicular side is $\sim 15,000$ A. A very small portion of this current, if shunted by the power line, can affect transformer cores considerably.

The observation–interpretation of the ssc-enhanced power harmonics leads to two points of interest: one scientific and one industrial. The scientific point concerns VLF chorus emissions. Chorus emissions are known to increase during magnetic substorms. This is generally understood in terms of the increased population of suprathermal electrons in the magnetosphere⁸. However, our investigation indicates that the enhancement of power harmonics during a magnetic storm may, itself, have an important role in giving rise to enhanced stimulation of emissions in the magnetosphere. The seeding of the harmonic waves is known to increase the emission activity^{9–12}, and our observation shows that the seeding process itself is enhanced during magnetic disturbances. Note that enhanced seeding of power harmonic waves means not only the increase in wave amplitude but also enrichment in spectral content, extension to higher frequencies and occurrence of even harmonics. The industrial point is that suitably distributed VLF receivers and induction magnetometers are expected to be useful for monitoring SICs in power systems. Observation of power harmonic waves by VLF receiver is expected to yield quantitative information on strength of SICs. SICs are likely to be caused by various types of magnetic variations: ssc, magnetic pulsations, polar substorm and magnetic storms. Identification of the particular type can be made through observation by induction magnetometers. Understanding the nature of SICs is expected to help find ways of minimising their undesirable effects on power generation, transmission and distribution.

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Thermoluminescence and terrestrial age of the Estacado meteorite

THE terrestrial age of a meteorite is the number of years which has elapsed since the meteorite fell to Earth. The terrestrial ages of most meteorites are not known directly, as most are 'finds' rather than observed 'falls'. Here we discuss the various methods which have been suggested to determine the terrestrial age of the 'finds', and these may be conveniently divided into two categories: (1) those which use the decay of spallation-produced radioactive isotopes^{1–4}; (2) those which use the thermal drainage of thermoluminescence (TL) from the material^{5,6}.

Boeckl¹ has calculated the terrestrial ages of 19 stony meteorites from a derivation of their radiocarbon content. Among the meteorites studied was Estacado (H-group chondrite) for which Boeckl calculated a terrestrial age of $6,800 \pm 3,000$ yr. Recently, McKeever and Durrani⁷ have carried out some TL studies of Estacado, the results of which are not consistent with such a large terrestrial age. After a meteorite falls, it is shielded from cosmic rays by the Earth's atmosphere. The TL induced in the meteorite by cosmic-ray bombardment in space will thus slowly drain from the meteorite as it rests on the Earth's surface, the high-temperature ($\approx 400^\circ\text{C}$) region of the TL glow decaying much more slowly than the low-temperature ($\approx 200^\circ\text{C}$) region. The high-temperature region of Estacado's TL glow seems to be far too high to be consistent with a terrestrial age of 6,800 yr.

Figure 1 shows the high-temperature (HT) TL of 14 meteorites of known terrestrial age (that is, 'falls') and the high-temperature TL of five 'finds'. The TL is plotted either against known terrestrial age, or against deduced age from ¹⁴C

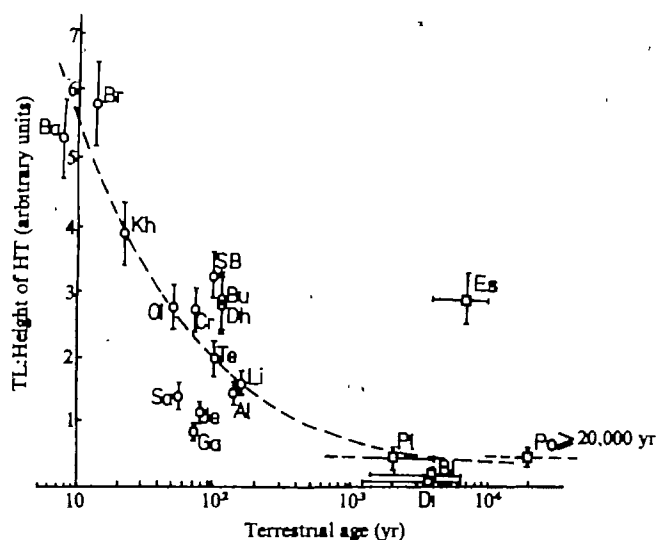


Fig. 1 The decay of the intensity of the high-temperature TL region from 14 'falls' (○) and five 'finds' (□). The ^{14}C age for Potter is from Suess and Wänke⁴. The uncertainty in the TL is estimated from the standard deviation of the high-temperature TL within a sample, based on 75 measurements of the natural TL in Estacado. The dotted curve is an estimation of the decay curve. Ba, Barwell; Br, Bruderheim; Kh, Khanpur; SB, Soko Banja; Bu, Butsura; Ol, Olivenza; Cr, Crumlin; Dh, Dhurmasa; Es, Estacado; Te, Tennesilim; Li, Limerick; Al, Aldsworth; Sa, Saratov; Je, Jelica; Ga, Gambat; Pi, Plainview; Bl, Bluff; Di, Dimmit; Po, Potter.

measurements^{1,4}. Clearly, Estacado is 'anomalous' in that the intensity of the high temperature TL is not consistent with a ^{14}C age of 6,800 yr. Indeed, the TL data suggest a true terrestrial age of 100 yr.

Further evidence is available to support a terrestrial age ≈ 100 yr for Estacado. First, its appearance is one of a reasonably 'fresh' fall. Besides rusting on the surface, the interior of the meteorite was very fresh looking (a pale grey) which, when the meteorite is compared with meteorites such as Dimmit or Potter, implies that it has a much lower terrestrial age than 6,800 yr.

Second, there is some strong circumstantial evidence to indicate that Estacado fell in 1882. Howard and Davison⁶ have given a detailed account of the find of Estacado. In 1882, a bright fireball was seen by the residents of the colony of Estacado, Hale County, Texas. The meteor was seen to fall north-west of them. In 1883 several cowboys came across the meteorite in the vicinity of the conjectured fall of the meteor. As this is a stoneless region, the inhabitants considered the meteorite to have been the cause of the fireball seen in 1882.

Viewed in the light of the account reproduced by Howard and Davison, the TL data clearly support the view that Estacado's terrestrial age is only ≈ 100 yr and not several thousands of years as deduced by Boeckl.

This discussion of Estacado's terrestrial age suggests that a useful check of a deduced ^{14}C age would be to carry out some TL measurements in order to check the consistency of the ^{14}C age with the TL properties. Similar inconsistencies have been highlighted in earlier work⁵.

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An explosive volcanic eruption in the Southern Hemisphere in 1928

SEVERAL climatologists^{1–3} have compiled lists of volcanic eruptions which are thought to be of meteorological and climatological importance. Although these lists have been criticised for being incomplete⁴, subsequent authors^{5,6} have used them in studies designed to reproduce and explain the climatic history of the Northern Hemisphere. I report here additional information on two volcanic eruptions which may be climatologically significant but have generally not been considered in climate studies. By clarifying the volcanic chronology, a better test of the hypothesis that volcanoes cause part of the observed climate change can be made.

From measurements of solar radiation by the Smithsonian Astrophysical Observatory made at Mt Montezuma, Chile, Mt Brukkaros, South-west Africa, and Table Mountain, California, there is evidence for an increase in atmospheric turbidity in 1928 which can be best attributed to a volcanic eruption which ejected aerosols into the stratosphere. Paluweh (8° 19'S, 121° 42'E) in the East Indies, which caused tidal waves with its eruption in 1928, is the major cause of the decrease in atmospheric transmission at this time, although Reventador (0° 05'S, 77° 40' W) in Ecuador which also erupted in 1929 may have contributed to this decrease. Radiation records at Washington, D.C., Madison, Wisconsin, Lincoln, Nebraska, and Blue Hill, Massachusetts also show decreases in atmospheric transmission⁷. Modern climatologists have generally overlooked the volcanic eruption of Paluweh in 1928, although this was probably one of the largest eruptions between that of Katmai in 1912 and Agung in 1963.

The Astrophysical Observatory of the Smithsonian Institution made pyrheliometric observations of the normal incidence irradiance of the Sun at Mt Montezuma, Chile (22° 40' S, 68° 56' W) from 1920 to 1955. These measurements were made with silver disk pyrheliometers and Angstrom pyrheliometers at selected air mass values. Many measurements were made at sea level air mass values of 2.0 and 2.5 and are tabulated by Abbot and Aldrich⁸ and Aldrich and Hoover⁹. The true air mass values are 1.44 and 1.80 for the mean station surface pressure.

The ratio of the pyrheliometric measurements at air mass 2.5 to that at 2.0 provides a relative measure of atmospheric transmission¹⁰ which is independent of the pyrheliometer calibration. Ratios are calculated on each day for which the pair of observations are available. Monthly means are calculated from the daily values. For those months with no data, the average value of all the months with the same name is used. The resulting time series is then smoothed by using a 13-month running mean average to remove seasonal variations. The resulting transmission curve is shown in Fig. 1.

Most of the features of this curve can be explained by known causes. The low transmission values before 1926 are caused by the volcanic eruption of Puyehue, Chile (40° 35' S, 72° 08' W) in December 1921 (refs 11, 12). The decrease in atmospheric transmission in 1932 was caused by the eruption of the Quizapu crater of Cerro Azul (35° 39' S, 70° 46' W) in Argentina in April 1932. A volcanic cloud 15 km in height was observed¹². This stratospheric event was observed throughout the Southern Hemisphere¹³ and ash fell over much of South America¹⁴. From Fig. 1 it can be seen that it took 2–4 yr for the atmosphere to return to normal transmission. This behaviour is characteristic of a violent volcanic eruption in which aerosols are injected into the stratosphere. After 1950 the atmospheric transmission decreases because of local contamination by a copper smelter at the Chuquicamata mine located north of the station. This local

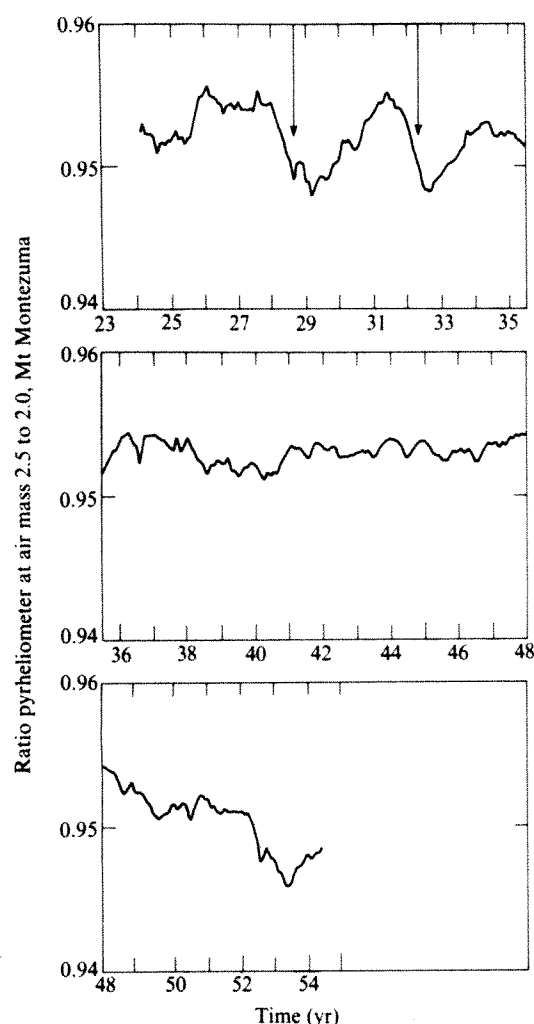


Fig. 1 The 13-month running mean values of the ratios of the pyrhelimeter measurements at air mass 2.5 to that at air mass 2.0 at Mt Montezuma, Chile. It is a measure of the relative atmospheric transmission. The increase in optical depth for air mass 1 after the eruption of Paluweh in 1928 averaged about 0.02 for 6 months after the eruption at Mt Montezuma. The times of the eruptions of Paluweh (1928) and Quizapu (1932) are indicated by arrows. Because running means are used, the apparent atmospheric transmissions seem to start decreasing about 7 months before the date of the eruption.

contamination becomes particularly evident in late 1952 with the opening of a major smelter.

Another decrease in atmospheric transmission is observed starting in 1928. The decrease and subsequent slow rise in atmospheric transmission is very similar to the event of 1932. It seems to be a stratospheric volcanic event in July or August 1928, but it is not mentioned in the usual lists of explosive volcanoes¹⁻³. The Smithsonian observations of the aureole showed an increase in brightness, and the pyrhelimetric measurements a decrease in intensity at this time in a manner similar to that in the period after the Quizapu eruption. There was no appreciable change in total precipitable water. The measured solar constants of the Smithsonian Astrophysical Observatory at Mt Montezuma, Table Mountain and Mt Brukaros also decreased at this time which is an indication of increased atmospheric turbidity because of errors in their reduction scheme¹⁵. Clearly, the decrease in atmospheric transmission in 1928 was caused by an increase in the stratospheric dust loading both because of the long period of decreased atmospheric transmission and because it was observed worldwide. Indeed, Störmer¹⁶ photographed stratospheric dust clouds in Europe in January 1929 which may have originated from the 1928 volcanic eruption.

Although several volcanoes erupted in the period 1927-29, the eruption of Paluweh in the Isle Flores or the East Indies was the primary cause of the increase in turbidity observed at Mt Montezuma. Paluweh was active from 4 August to 25 September 1928 (ref. 17) and was the only violent volcanic eruption at this time. The eruption was accompanied by a tidal wave and the creation of three craters with diameters 80-600 m wide. The decrease in atmospheric transmission at Mt Montezuma was observed immediately after this eruption and continued for about 2 yr.

Reventador (0°05' S, 77°40' W) in Ecuador erupted in 1929 with ash rains evident in the Andes¹⁸. This eruption may have prolonged the decreased atmospheric transmission and may account for the double-bottomed depression in Fig. 1. Other minor eruptions in 1928 were Slamet (7°14' S, 109°12' E) in Java, Bromo (7°56' S, 112°57' E) in Java, Raung (8°07' S, 114°02' E) in Java, Ili Werung (8°32' S, 123°35' E) in the Lesser Sunda Islands, and Batuk Petarangan (7°10' S, 109°49' E) in Java. Active volcanoes in 1929 include Quizapu, Mt Pelee on Martinique, and Asama in Japan.

Hand⁷ independently confirms that Paluweh was the major contributor to increased turbidity in 1928 and 1929 using the Weather Bureau pyrhelimeter data. His brief mention of an eruption in the Isle Flores, Dutch East Indies, seems to be the only previous comment on a volcanic eruption at this time in the meteorological literature. At Washington, D.C., Madison, Wisconsin, Lincoln, Nebraska, and Blue Hill, Massachusetts, the atmospheric transmission decreased beginning in April of 1929. The Southern Hemisphere autumn is the most favourable time for the transport of stratospheric aerosols across the Equator^{2,19}, hence the delay of 6-8 months of the effect of the eruption of Paluweh on the radiation records in the US. Meinel and Meinel²⁰ found a comparable delay before volcanic dust from the Agung eruption became evident over the US; Paluweh and Agung are located close to each other. From the Smithsonian records, the Lamb dust veil index for this volcano seems to be about 100, thus making it the most important eruption between Katmai in 1912 and Agung in 1963. The decrease in atmospheric transmission in the US after the Paluweh eruption was about 30-40% of that observed after the Agung eruption.

The eruptions of Paluweh in 1928 and Quizapu in 1932 may have had a worldwide effect on the mean surface temperature, as indicated by Fig. 2 taken from Oliver⁵. One year after both eruptions there was a decrease in the mean surface temperature of the Northern Hemisphere of the order of 0.3 °C. Miles and Gildersleeves²¹, have shown that 1 yr after a major volcanic eruption there is on the average a decrease in the mean surface temperature of 0.29 °C and that there is only 'a small probability of the result being due to chance.' Mass and Schneider⁶ find that the depression in surface temperatures 1 yr after a volcanic eruption is significant at better than the 99% confidence level. Humphreys²², Mitchell²³, Yamamoto *et al.*²⁴, and others reach similar conclusions based on experimental data, and Hansen *et al.*²⁵, Baldwin *et al.*²⁶, and others support these observations by theory. Thus, there is reason to believe that the depressed surface temperatures in 1929 and 1933 may have a cause and effect relationship with the volcanic eruptions occurring 1 yr earlier. These drops in temperature may occur by chance but the probability of this happening is low, probably less than 1%. From Budyko²⁷, the year-to-year variation in the Northern Hemisphere annual mean surface temperature is 0.12 ± 0.097 °C for the period 1923-54. Therefore, the probability of a 0.3 °C temperature change being caused by chance alone is about 1% or less using three standard deviations as a guide. Furthermore, the probability of successfully identifying the years when the temperature decreases will occur only from a knowledge of the dates of the major volcanic eruptions is even less. In short, there is a strong suggestion that the volcanic eruptions affect climate 1 yr afterwards but not longer. Further studies of these eruptions and their effect on atmospheric transmission and climate will be needed to confirm these conclusions.

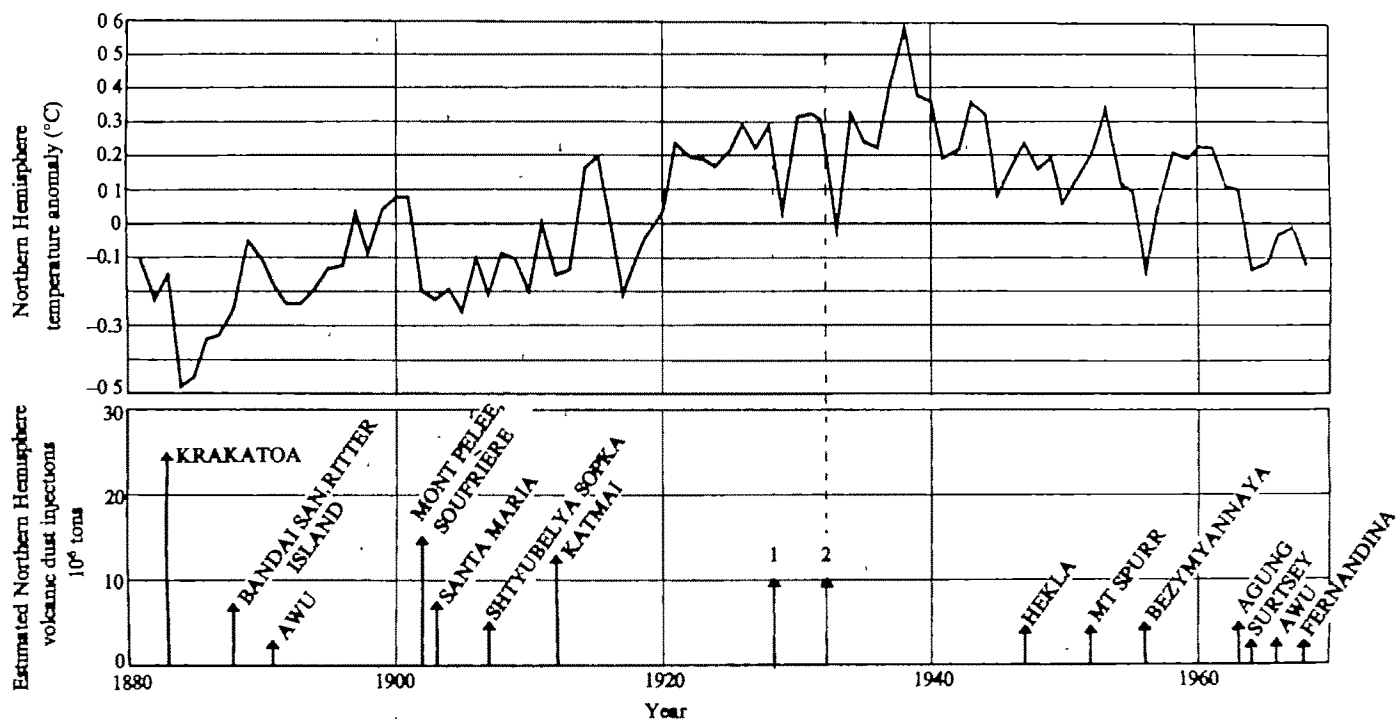


Fig. 2 The Northern Hemisphere temperature anomaly ($^{\circ}\text{C}$) as given by Oliver⁵. Major volcanic eruptions are indicated below. Events 1 and 2 correspond to the volcanic eruption of Paluweh in 1928 and to the Quizapu eruption in 1932. These Southern Hemisphere volcanic events may have affected the Northern Hemisphere temperatures, as can be seen by the decrease in temperatures about 1 yr after the two events. (The arrows for Paluweh and Quizapu are primarily used to indicate the times of their eruptions, not their strength.)

Most climatologists¹⁻³ have failed to include these eruptions in their lists and have included other volcanic eruptions which do not show up in the radiation records. For example, Oliver⁵ omits these eruptions in his analysis of the Northern Hemisphere surface temperatures, but it seems that volcanic dust originating in the Southern Hemisphere can cross the Equator and significantly affect the climate in the Northern Hemisphere. However, we emphasise that not all features of the climatic record can be explained by volcanic eruptions, although certain fluctuations in climate do seem to be caused by this mechanism. The climatology of the Southern Hemisphere is not yet well enough established in the 1920s to determine the effect of the eruption of Paluweh on the climate of the Southern Hemisphere.

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Stimulated resonance between electrical and shear fields by a colloid system

CERTAIN polymer solutions have been shown using a Couette shear dielectric cell¹ to exhibit changes in dielectric properties when subjected to a shear field^{2,3}. For polar polymers which lack flexibility, the action of a rotational shear field alters the time-dependent distribution of dipole orientation which results in changes of permittivity, loss magnitude and relaxation frequency². In quantitative terms these changes seem to correspond well with the theoretical treatment due to Barisac⁴. A distinct difference in the situation occurs when flexible polymers such as poly(styrenes) or poly(*p*-ethoxystyrenes) are subjected to such shear fields. The changes are inconsistent with the magnitude of the polymer dipole and solvent relative permittivity takes on a rather dominant role⁵. To test the importance of variation of the internal field term on shape changes due to polymer coil distortions, we have studied an organic colloid system: cross-linked poly(ethylacrylate) dispersed in heptane and stabilised with a 'comb' graft copolymer [poly-(12-hydroxy stearic acid) terminated with glycidyl methacrylate and then copolymerised (1:1) with methyl methacrylate]⁶.

This latex system consists of rubbery particles of 0.4 μm average diameter which can presumably shear distort. We observed a resonance (Fig. 1) between the shear field (shear rate G (s^{-1})) and electric field f (Hz). We know of no previous observation of such a phenomenon.

Certain preliminary observations suggest a mechanism for the effect. A study of the frequency dependence of relative permittivity (ϵ') and loss (ϵ'') in the absence of a shear field indicates, as shown in Fig. 2, that the heptane dispersed colloid has a high relative permittivity at low frequency and that at least two loss

processes are present. One occurs with a maximum at such a low frequency ($<10^2$ Hz) that its resolution is not possible with our audio frequency bridge equipment; the other apparently occurs at a frequency $\sim 10^3$ Hz as indicated by spot coaxial line and wave guide measurements. Even attempts to shift the low frequency process completely into the range of measurements ($>10^2$ Hz) by increasing the temperature to 60°C failed, though a partial shift did take place. A further temperature increase was not practicable due to solvent volatility. The large value of the relative permittivity at 10^2 Hz of a 40% dispersion above that of pure heptane (Fig. 2) together with the presence of two loss

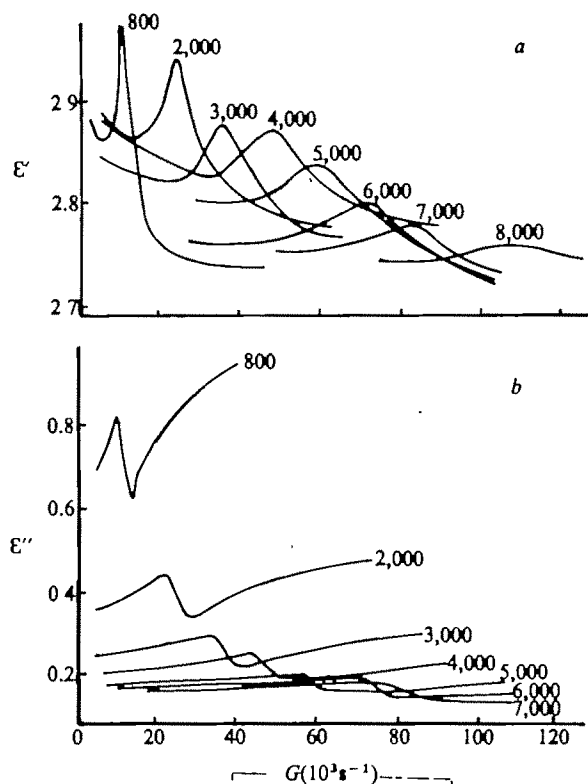


Fig. 1 Resonances in relative permittivity (ϵ') (a) and dielectric loss (ϵ'') (b) for the organic colloid in shear (rate G (s^{-1})). Numbers give polarisation frequency f (Hz).

processes at vastly different frequencies suggest that dissimilar mechanisms are responsible. The high frequency process is probably dipolar whereas that at low frequency is in our view interfacial (Maxwell-Wagner), although a dipolar origin in the poly(ethylacrylate) cross-linked phase cannot be ruled out. We favour an ionic-conductance process due to trace impurity ions, through or over the surface of the particles, promoting interfacial polarisation rather than orientation polarisation because of its low frequency, large magnitude and its agreement with a mechanism described below. Also, at the frequency of this process a bulk conductivity term is present shown by values of ϵ'' exceeding the incremental relative permittivity ($\Delta\epsilon'$).

On the basis of an interfacial polarisation the resonances shown in Fig. 1 originate in a coupling between electric field-induced charge displacement and forced rotation of the particles by the shear field. Indeed, the condition for the position on the shear-rate axis for such maxima (G_{max}) can be predicted to occur when the angular frequencies of polarisation and rotation match. The condition that $2\pi f = G_{\text{max}}/2$ follows if the particles are spherical and rotational diffusion has no influence⁶. Angular fluctuations due to brownian motion for such large particles will be small and even if significant, are not likely to alter the position of the maximum. The neglect of diffusion is therefore justified. It follows that f should be proportional to G_{max} and this is shown in

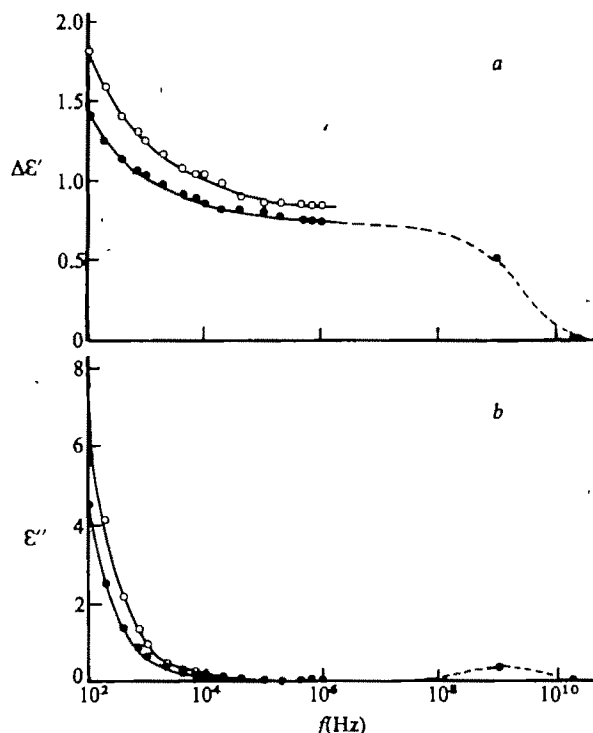


Fig. 2 The increment in relative permittivity ($\Delta\epsilon' = \epsilon'_{\text{solution}} - \epsilon'_{\text{solvent}}$) (a) and dielectric loss (ϵ'') (b) of the organic colloid in heptane. $\bullet$, 30° ; $\circ$, 60° . Dashed line is interpolation based on limited data.

Fig. 3. Also shown in Fig. 3 is the theoretical line of slope $(4\pi)^{-1}$. The slight discrepancies we attribute to inaccuracies in gap measurement. An error of $1.3 \mu\text{m}$ in the gap measurement of $23 \mu\text{m}$ would account for the divergent slopes: this is within the limits of the capacitance based measurement¹.

Work is proceeding on this resonance phenomenon and its application to aqueous media. This important class of polymer systems, which includes biopolymers in their natural environment, presents considerable problems in dielectric studies in the kHz-MHz frequency range because of the screening effect of bulk conductance. We have experimentally established with dioxan-water mixtures that bulk conductance in isolation does not give rise to a shear-induced resonance in permittivity. Thus, this resonance phenomenon may possibly be used to decouple

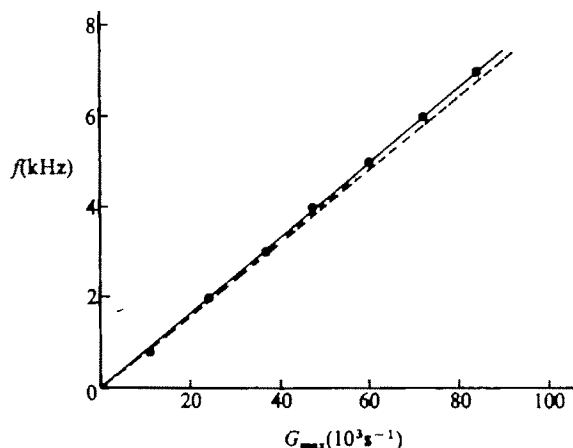


Fig. 3 The dependence of shear rate at maximum resonance (G_{max}) on f . The theoretical dependence of slope $(4\pi)^{-1}$ is shown as a dashed line.

dielectric relaxations, particularly interfacial ones, from the screening effect of bulk conductivity.

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Epitaxial synthesis of diamond by carbon-ion deposition at low energy

THIN epitaxial layers have been grown in vacuum by the controlled deposition of low-energy carbon ions on to heated single crystal diamond substrates by a process described here. Reproducible crystalline growth, up to 10 μm in thickness and up to several mm^2 in area, has been observed on both natural stones and polished diamond plaques¹. Three competing forms of ion-deposited carbon have also been characterised.

Various methods of diamond synthesis have been reported; the most successful involve growth at elevated temperature under very high pressure. Limited success has also been achieved in the metastable region by vapour-phase epitaxy at low pressure², but such procedures are constrained by the spontaneous graphitisation of diamond at high temperatures. At the lower temperatures which have been used, the scale and the quality of crystalline growth are severely limited by the competing formation of other modifications of carbon.

In 1971, Aisenberg and Chabot³ described the production of 'diamond-like' films by the deposition of low-energy carbon ions on a variety of surfaces. The depositions were carried out at room temperature and the substrates did not include diamond. The further characterisation of such films by ellipsometry and X-ray and electron diffraction has been reported by Spencer *et al.*⁴. Although the properties of the deposits were variable, there was evidence of polycrystallinity with definite, but usually very weak, X-ray lines assignable to diamond.

In 1967 J.H.F. described the adaptation of a heavy-ion accelerator for a variety of ion-bombardment studies on semiconductor substrates⁵. These included the study of epitaxial growth through the deposition of low-energy silicon ions on heated wafers of single crystal silicon. Such epitaxy is to be expected and it simply reflects the thermodynamic stability and the established vapour-phase growth behaviour of crystalline silicon. It has been subsequently confirmed in more detailed studies⁶ using low-energy silicon and germanium ions. The situation with respect to carbon is quite different; not only is diamond metastable in such deposition conditions, but it has also been observed that the radiation damage due to ion bombardment can result in irreversible graphitisation⁷.

Nevertheless, some encouraging indications of diamond synthesis were obtained in an initial feasibility study carried out in 1972. Carbon ions with an energy < 100 eV were deposited on to a small area of a heated diamond plaque. The resulting brown layer, ~3,000 Å in thickness, resisted simple abrasion

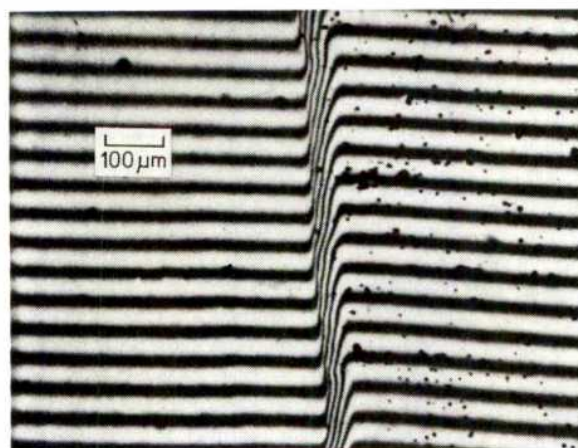


Fig. 1 Interferometric micrograph of an epitaxial deposit on a polished diamond plaque. Note the carbonaceous inclusions in the deposited layer.

tests and showed evidence of crystallinity when studied by proton channelling. It thus seemed that the very localised dissipation of energy as the ions were stopped on, or very close to, the surface may have assisted epitaxial growth by inducing mobility at an acceptable substrate temperature.

A wide range of experiments was carried out subsequently on a much improved ion-deceleration apparatus⁸ installed on a Harwell/Lintott type of heavy-ion accelerator. These demonstrated that the deposition process is complex and that at least three other forms of carbon can compete with the epitaxial synthesis. It was also found that, at low ion energies, even slight surface contamination affects the reproducibility of deposition. The most consistent results and the best quality of growth were obtained using carbon ions at an energy of around 900 eV on carefully cleaned diamond plaques, heated to about 700 °C. The layers, which were deposited to a thickness of several micrometres at a rate of a few thousand Ångströms per hour, regularly contained a low density of carbonaceous inclusions but otherwise apparently represented continuous epitaxial growth on the crystalline substrate.

An interferometric micrograph of an epitaxial deposit containing inclusions on a polished diamond plaque is shown in Fig. 1. Evidence of crystallinity in such a layer can be seen in the reflection high-energy electron diffraction pattern (RHEED) in Fig. 2. Various other techniques were also used to characterise the ion deposited layers. Finally, on the basis of the accumulated experience with a large number of samples, it was concluded that non-epitaxial carbon could be confidently distinguished and removed by extended polishing with alumina powder followed by controlled air oxidation for periods up to two weeks. The epitaxial growth was totally resistant to such treatment.

It was found that the density of the carbonaceous inclusions could be substantially reduced by rigorous precleaning of the substrate but they were never completely eliminated. They seem to be a lower density form of carbon than diamond and they consequently grow steadily in size as the deposition proceeds. It was thus found that they provide an ultimate limit to the

Fig. 2 Reflection high-energy electron diffraction (RHEED) pattern of an ion-deposited epitaxial layer.



thickness of crystalline growth which can be achieved in a single stage.

In some experiments a continuous smooth deposit with a lower reflectivity and density than diamond was observed in place of the epitaxial growth. The nature of these carbonaceous layers which may have been associated with the use of a conventional oil-pumped vacuum system, varied markedly from sample to sample. Additionally, a matt black form of carbon was sometimes observed at the edge of deposits when masks were used to define the boundary of growth. A fuller account of this work will be published elsewhere.

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The role of hydrogen sulphide in environmental transport of mercury

HYDROGEN SULPHIDE may be evolved in anoxic sulphur-containing river or lake sediments¹. The effect of the presence of sulphide on the availability of inorganic mercury for biological methylation has been considered previously. Essentially mercury in the form of Hg(0) or Hg(II) may be converted into mercuric sulphide in anoxic sediments². It might seem that this very insoluble material would not, in practice, be available for methylation and that sulphide formation would act as a method of removing mercury from availability to the general environment (that is, it would act as a mercury sink). However in a complex natural system (aquarium sediment) it has been shown that HgS may, in fact, be methylated in the sediments although to produce quantities of methyl mercury substantially less than those produced using a more soluble mercury substrate². Therefore, although H₂S in a sediment would be expected to reduce the rate of mercury methylation considerably by the formation of HgS, it would not be expected to prevent formation of methyl mercury entirely. The effect of H₂S on methyl mercury itself has also been investigated using a purely inorganic model³. An aqueous solution of methyl mercuric chloride (1 mg dm⁻³) loses methyl mercury steadily over 3 days after initial exposure to H₂S. A volatile product was evolved into the space above the solution. This product was trapped in a sodium carbonate-disodium phosphate solution, extracted with toluene and shown to co-migrate with methyl mercury on thin layer plates of silica gel. It was concluded that the evolved product of the reaction system was a volatile organo-sulphur derivative of mercury that reverted to methyl mercury on extraction. These observations suggest that reaction between methyl mercury and H₂S generated in sediments reduces the amount of methyl mercury available for accumulation by fish and shellfish and represents a

powerful mechanism adding to the general mobility of mercury compounds in the environment. It is therefore important to identify the unknown mercury compound evolved from the system described above. We have now investigated the interaction of H₂S with River Mersey (England) inter-tidal estuary sediment samples in order to determine its effect on methyl mercury levels in real systems. We have also investigated similar reactions in a model system, leading to the identification of the previously unknown volatile mercury species responsible for the transport of mercury in the work of Rowland *et al.*³

Our experiments with the model system were similar to those described previously³, but we used a gas chromatographic technique rather than radiochemical labelling to estimate methyl mercury losses. Aqueous solutions of CH₃HgCl (concentration about 1 mg dm⁻³) were placed in screw top bottles fitted with silicone rubber septa. H₂S or N₂ (for control samples) was passed through the solutions for 2 min. At intervals solution samples were extracted by syringe from each bottle and analysed for methyl mercury as follows.

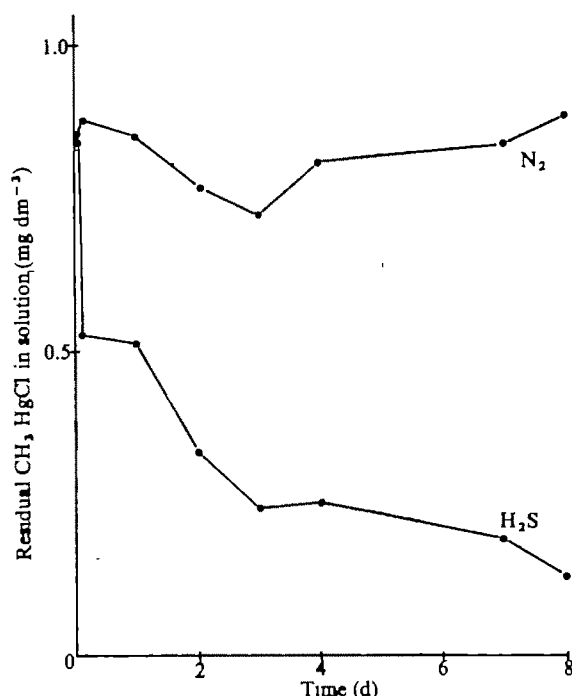


Fig. 1 Volatilisation of CH₃HgCl from aqueous solution by H₂S. Initial concentration of solutions was 1 mg dm⁻³. There was an immediate rapid decline to 0.85 mg dm⁻³ for both solutions owing to adsorption on vessel surfaces. This initial decline over the first few minutes of the experiment has not been included in the graph. Temperature, 18 °C.

An aliquot of 0.1 cm³ of each solution was placed in a tube and N₂ gas passed over the solution for 30 s to remove excess H₂S. (CH₃HgCl is not affected by this procedure.) The sample solution was then shaken with 1 cm³ benzene (GLC grade) and 0.1 cm³ CuSO₄ solution (10%) for 2 min. The benzene layer was removed and the methyl mercury content determined by electron-capture gas chromatography as described previously⁴. We found that methyl mercury was steadily lost from the original solution and a typical example from an experiment of this kind is shown in Fig. 1. These results are not incompatible with those of Rowland *et al.*³ although our experiments were conducted over a rather longer period.

As the gas chromatographic technique used was probably specific for monomethyl mercury, an attempt was made to identify any other species in the solution using ¹H NMR spectroscopy. The NMR spectrometer used (Jeol C60-HL) could detect methyl mercury in concentrations of >0.5%. Because of solubility difficulties with the chloride we carried out NMR experiments using methyl mercuric acetate at a concentration of

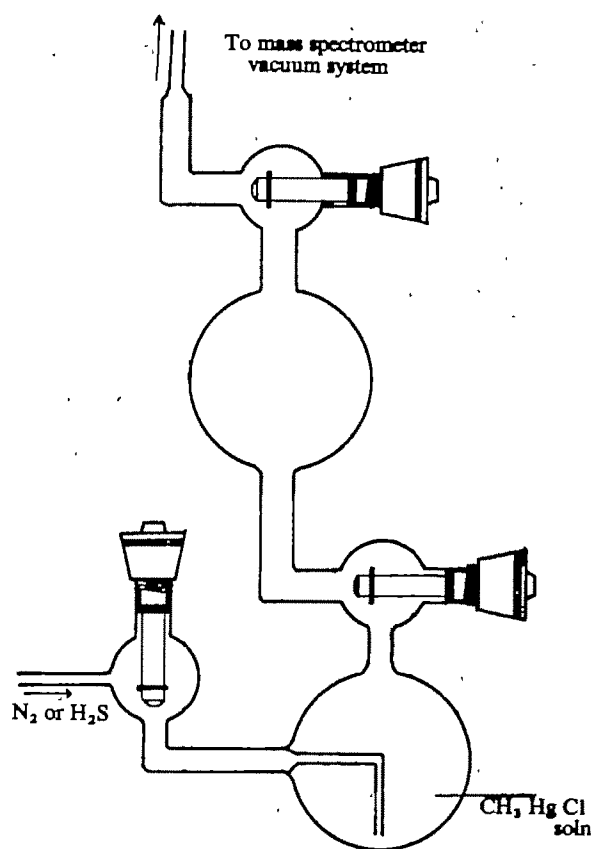


Fig. 2 Two chamber vacuum vessel. Apparatus designed for connection to mass spectrometer vacuum line. (Taps used: glass/PTFE—J. Young, London.)

1% in D_2O . NMR spectra of $CH_3HgOCOCH_3$ in D_2O show three principal peaks for methyl mercury, acetate-methyl and residual water at 9.0, 8.0 and 4.3 τ values respectively. For the H_2S experiment, 0.25 cm³ of 2% $CH_3HgOCOCH_3$ solution in D_2O was mixed with 0.25 cm³ of saturated H_2S solution in D_2O in an NMR tube (a white precipitate formed immediately). The tube was then sealed and a spectrum recorded. The NMR spectrum showed peaks only for acetate-methyl and H_2O . The H_2S peak did not appear owing to proton exchange with D_2O . The absence of the methyl mercury peak shows that the formation of the white precipitate has reduced the methyl mercury concentration in the solution to below that detectable by the NMR technique.

The control (no H_2S) and the experimental sealed NMR tubes were re-examined several times over a 14-day period. No changes in the spectra were seen over this period. The white precipitate, however, did change colour slowly from white to black.

The initial white compound was prepared, separately, from H_2S solution and $CH_3HgOCOCH_3$. After recrystallisation from ethanol, the melting point was found to be 142 °C at which temperature the compound decomposed to a black solid. A mass spectrum was run of the white compound whose highest m/e values and characteristic mercury isotope pattern (see for example ref. 5) and distribution demonstrated it to be $(CH_3Hg)_2S$. The melting point is consistent with the literature melting point (143 °C) for $(CH_3Hg)_2S$ (ref. 6).

It seems then that loss of methyl mercury from aqueous solution on exposure to H_2S is caused initially by formation of $(CH_3Hg)_2S$. The solutions used by Rowland *et al.* and in our GLC experiments were much more dilute than the solutions used for NMR, and this might account for a slower appearance of $(CH_3Hg)_2S$ in our GLC work. The subsequent change of

white $(CH_3Hg)_2S$ to a black material represents a further reaction that may account for the actual volatilisation and loss of mercury from the system.

An experiment was devised to try to identify the volatile product(s) by mass spectrometry. A two-chamber vessel (Fig. 2) was constructed such that the aqueous phase reaction between CH_3HgCl and H_2S could be carried out with subsequent separation of any volatile products. Twenty-five cm³ of aqueous CH_3HgCl (1 g dm⁻³) were placed in the lower section and N_2 (control experiment) or H_2S was passed through the solution for 2 min. With H_2S a white precipitate formed immediately. After 3 days in the closed system the upper chamber was evacuated and any volatiles present in the lower chamber were allowed to pass into this upper section. The apparatus was connected to a vacuum line system attached to a mass spectrometer (V.G. Micromass 16B). The contents of the upper vessel were trapped out using liquid nitrogen in a section of the vacuum system. After initial warming excess H_2S was pumped away and the trap contents were allowed to enter the mass spectrometer. The characteristic mass spectrum of dimethyl mercury was observed. The compound was identified by the characteristic isotope pattern observed for mercury⁵ surrounding m/e 232, 217 and

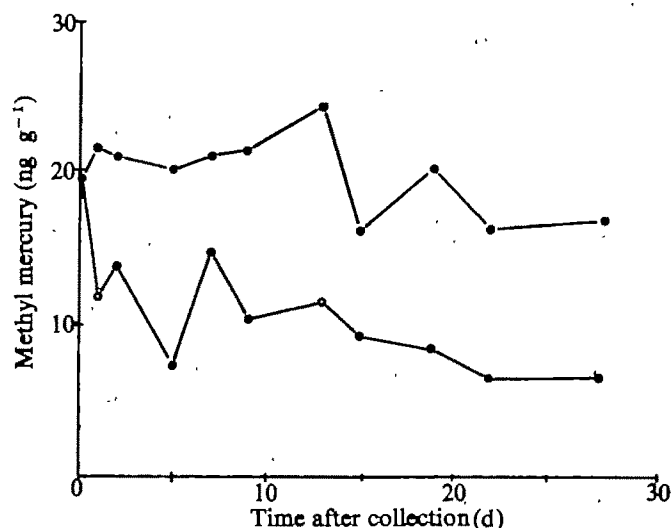


Fig. 3 Analysis of sediment samples (Hale Point, Mersey Estuary) for methyl mercury. Samples stored at room temperature (18 °C). ●, Untreated; ○, H_2S treated samples.

202 corresponding to $(CH_3)_2Hg^+$, CH_3Hg^+ and Hg^+ . The control sample did not evolve observable amounts of CH_3HgCl into the vapour phase and hence we conclude that our observed CH_3Hg^+ and Hg^+ peaks were caused by breakdown of $(CH_3)_2Hg$ not evolution of CH_3HgCl . In any case we observe no peaks corresponding to m/e 254 and 252 for CH_3HgCl , nor any $HgCl^+$ species at 239 and 237. This evidence suggested that only $(CH_3)_2Hg$ had been evolved. We could find no evidence for CH_4 or any mercury sulphur species. Comparison with standard mass spectra of known samples of $(CH_3)_2Hg$ and CH_3HgCl reinforced these conclusions. We could find no evidence for the presence of suitable mass ratio peaks for compounds such as CH_3HgSCH_3 and CH_3HgSH . The H_2S experiment was repeated using a 1 mg dm⁻³ solution of CH_3HgCl but this was too low a concentration for detection in our apparatus.

The results of this series of experiments suggest that Rowland *et al.*³ observed the evolution of volatile $(CH_3)_2Hg$ from the reaction between H_2S and CH_3HgCl . $(CH_3Hg)_2S$ was apparently formed as an intermediate in the reaction. This compound gradually decomposes to black β - HgS and $(CH_3)_2Hg$. This is in accord with the known chemistry of this species^{7,8}.

Our results for CH_3HgCl levels in solution differ in small detail from those of Rowland *et al.* who measured total CH_3Hg species remaining in solution, whereas our technique measures water soluble CH_3Hg species remaining in solution. This would include CH_3HgCl and possibly, to a small extent, $(\text{CH}_3\text{Hg})_2\text{S}$. In our experiments a precipitate of $(\text{CH}_3\text{Hg})_2\text{S}$ was observed. Rowland *et al.* do not mention this and it is likely that at the higher temperatures they used (37°C) a precipitate was either not present or present only to a very small extent. Our experiments were carried out at 18°C .

The rapid early loss of methyl mercury found in our experiments is probably due to initial rapid formation of some $(\text{CH}_3\text{Hg})_2\text{S}$ to an equilibrium level, followed by a slower decay of the sulphide to dimethyl mercury which is lost from solution.

The question arises as to how much significance these reactions have for environmental situations. In our experiments on the analysis of estuarine sediments for methyl mercury we have observed changes in methyl mercury levels over a number of days following sampling⁴. Changes in the redox conditions of the sediment following sampling were suspected as a possible cause of this effect. In a series of such experiments we passed H_2S through a container of freshly-sampled sediment; the sediment was later sub-divided into a series of bottles. Single bottles were analysed for methyl mercury over 28 days and compared with a control set which was not exposed to H_2S .

These experiments have demonstrated that diffusion of H_2S in a sediment significantly reduces the level of methyl mercury in that sample relative to a control (Fig. 3.). We have also been able to show that dimethyl mercury is evolved into the gaseous phase from a mercury-containing sediment which has been treated with H_2S . A control sediment not exposed to H_2S did not evolve $(\text{CH}_3)_2\text{Hg}$ in detectable quantities. For this latter experiment we were forced to raise the concentration of methyl mercury to $100\text{ }\mu\text{g g}^{-1}$ by 'spiking' with CH_3HgCl . At natural concentrations of methyl mercury, of the order of 15 ng g^{-1} , we could not detect $(\text{CH}_3)_2\text{Hg}$ in control or H_2S exposed samples.

Our results suggest that monomethyl mercury species in sediments may be mobilised into the atmosphere as $(\text{CH}_3)_2\text{Hg}$ if H_2S is generated in the sediments. This points to a natural mechanism for the removal of mono-methyl mercury from situations where it may be incorporated into fish. In positively identifying $(\text{CH}_3)_2\text{Hg}$ as the volatile species another route in the general mercury cycle has been demonstrated. In view of the potential generation of further monomethyl mercury species from the large 'reservoir' of inorganic mercury that accompanies monomethyl mercury in sediments⁹, there is the possibility of volatilisation of larger amounts of mercury than the equilibrium amounts of methyl mercury present.

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Reevaluation of the role of dimethyl sulphide in the sulphur budget

THE possible existence of an oceanic source of atmospheric SO_2 has recently been suggested for the following reasons. First, to account for the observed¹ SO_2 background concentration of $0.1 \times 10^{-6}\text{ g m}^{-3}$ of air, even very far from any continental source and in areas as remote as the Antarctic ocean—despite a residence time for SO_2 in the marine atmosphere of $\sim 0.7\text{ d}$ (refs 2, 3). Second, because another major source such as the ocean is necessary to complete the global atmospheric sulphur budget⁴⁻⁷. Because of the basic pH of seawater, SO_2 cannot be emanated directly from the oceans. According to Lovelock *et al.*⁸, the dimethyl sulphide (DMS) produced in the sea by many living systems, could be evolved in a first step into the atmosphere and subsequently oxidised to SO_2 . These authors observed a mean DMS concentration of the order of $12 \times 10^{-9}\text{ g l}^{-1}$ in Atlantic ocean water. We have developed an improved method for the measurement of DMS in seawater and have used this technique on several recent oceanographic research cruises. Our results suggest that the oceans could contribute over 30% of the amount of sulphur required to balance the sulphur budget.

DMS was first extracted from the seawater using carbon tetrachloride, then by 5% aqueous solution of mercuric chloride as a Hg^{2+} -DMS complex which remains stable for more than 6 months. In the laboratory, DMS was released by 6 M HCl, redissolved in propan-2-ol at -35°C and finally measured by gas chromatography with a flame photometric detector. The limit of detection is $5 \times 10^{-11}\text{ g}$ of DMS. With the usual yields, this figure corresponds to a limit of $5 \times 10^{-9}\text{ g l}^{-1}$ for a 15-l sample of seawater.

A preliminary list of DMS concentrations for the Atlantic and Indian Ocean waters is shown in Table 1. The lowest values found agree well with the results of Lovelock *et al.*⁸. The highest concentrations observed (samples 1, 4 and 12) characterise areas with presence of algae (principally of *Fucus* and *Ascosiphium* with *Polysiphonia Lanosa*). Sample 2 was taken in water highly polluted by oil, 1 week after the oil spill of the Amoco Cadiz.

We determined levels of DMS in the microlayer and the concentration distribution with depth in the Mediterranean. To the best of our knowledge, this is the first report of such measurements. Surface microlayer samples (about $400\text{ }\mu\text{m}$) were obtained by using the Garrett screen⁹. The DMS concentrations found for these samples are given in Table 2.

As a general rule, the values observed in the Mediterranean are significantly higher than in the Atlantic. The concentrations

Table 1 Concentration of DMS in Atlantic and Indian Ocean seawater

Sample no.	Location	Date of sampling	Concentration (10^{-9} g l^{-1})
1	Northern coast of Brittany	February 1978	38 ± 8
2	Portsal (Brittany)	March 1978	19 ± 4
3	Roadstead of Brest	March 1978	7 ± 1
4	Ré island	May 1978	23 ± 5
5	Ré island	May 1978	11 ± 2
6	Ré island	May 1978	15 ± 3
7	Oleron island	May 1978	11 ± 2
8	Oleron island	May 1978	13 ± 3
9	Oleron island	May 1978	13 ± 3
10	Oleron island	May 1978	24 ± 5
11	Guinea Gulf ($2^\circ 35'\text{S}$ – $3^\circ 02'\text{E}$)	June 1977	14 ± 3
12	Amsterdam island ($37^\circ 47' 30''\text{S}$ – $77^\circ 32'\text{E}$)	March 1977	34 ± 7

Table 2 Concentration of DMS in Mediterranean seawater

Sample no.	Date	Location	Concentration (10^{-9} g l^{-1})		Sea state and temperature of surface water
			Microlayer	Subsurface	
13	28 June 1977 16 h–18 h	7°30' E 41°20' N	1,500 ± 300	580 ± 116	Calm
14	29 June 1977 15 h–16 h	7°30' E	800 ± 160	150 ± 30	Calm
15	5 April 1978 14 h 30–15 h 30	7°48' E 43°17' N	70 ± 14	60 ± 12	Calm 13 °C
16	6 April 1978 10 h 30	7°23' E 43°38' N		60 ± 12	Rough 13 °C
17	7 April 1978 10 h	7°24'30" E 43°36'30" N		51 ± 10	Rough 13 °C
18	26 April 1978 15 h 30	43°22' 7°40' E		17 ± 3	Rough 14.70 °C
19	27 April 1978 10 h	43°36' N 7°25' E		15 ± 3	Rough 14.80 °C
20	28 April 1978 13 h	43°22' N 7°44' E		11 ± 2	Rough 13.80 °C

measured in samples 13 and 14 seem to be anomalously high. Possible contamination during the sampling can be ruled out, however, because of the strict precautions taken (for instance, the water was sampled from a small dinghy 1 km forward of the ship). The microlayer is more concentrated in DMS than the subsurface water, with ratios of microlayer and subsurface

levels of between 1.2 and 5.3. Similar ratios (~ 3) have been observed for particulate organic carbon and nitrogen in the microlayer^{10–13}.

The DMS depth profiles obtained are shown in Fig. 1 together with those of temperature, salinity and density of the seawater. These profiles show the existence of high DMS concentration layers under the sea surface. The maximum DMS concentrations seem to be connected with a sharp variation in the gradient of density and therefore to a dynamically stable water layer. A similar observation is usual in the case of marine particulate matter.¹⁴

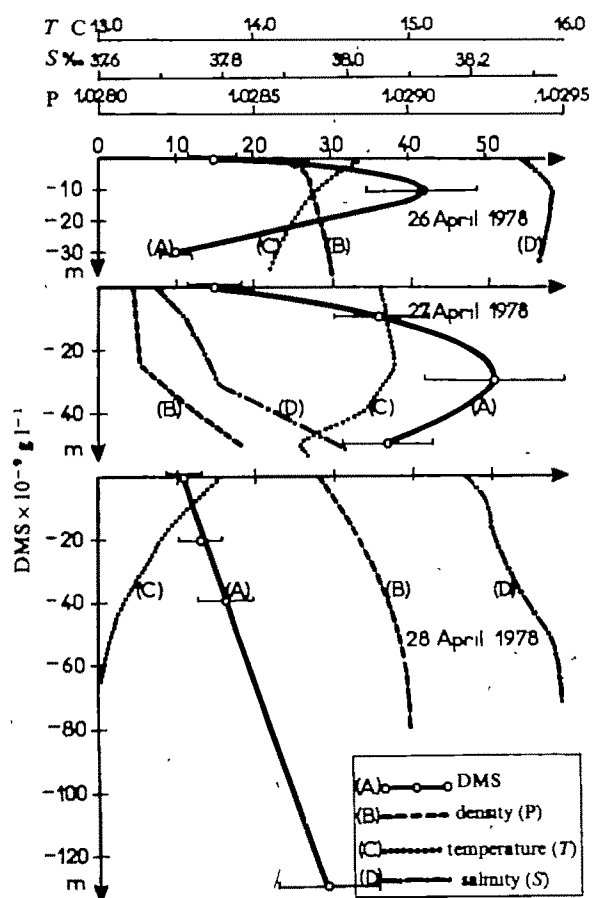
From these preliminary studies, we can draw several conclusions. First, a very large range of DMS concentrations is observed in the superficial seawater, the figures previously obtained by Lovelock⁸ appearing to be a lower limit rather than typical of the average oceanic value. Second, the DMS concentration also varies quite rapidly with depth: factors of 3 have been observed, as in Fig. 1, between the surface and the depth of the maximum concentration (10 and 30 m). These maximum DMS concentration layers seem to be able to move up, and to reach the surface, which could explain the existence of sometimes very high surface DMS concentrations. Both of these observations suggest that the mean oceanic DMS concentration should be of the order of $30 \times 10^{-9} \text{ g l}^{-1}$ instead of $12 \times 10^{-9} \text{ g l}^{-1}$ indicated by Lovelock⁸. Third, from the knowledge of the oceanic content of DMS, it should be possible to evaluate the flux of this sulphur compound to the atmosphere. Liss and Slater¹⁵ have suggested a method of calculation based on Fick's law of molecular diffusion and a Henry's law constant of 0.3:

$$F = K_1(C_{\text{air}} - C_1)$$

where F is the DMS flux ($\text{g cm}^{-2} \text{ h}^{-1}$), K_1 the exchange constant equal to 19.2 cm h^{-1} , C_{air} and C_1 respectively the DMS concentration in the air (negligible¹⁶) and in the seawater immediately under the molecular diffusion layer, that is here the microlayer concentration.

According to the present work, $C_1 > 90 \times 10^{-9} \text{ g l}^{-1}$ which is 3 times the mean subsurface concentration, since the sampling method of the microlayer partially mixes the superficial film with the subsurface water. This formula gives for the oceans a flux of at least $2.7 \times 10^{13} \text{ g yr}^{-1}$ of sulphur. Such a flux could represent more than 30% of the amount needed to balance the sulphur budget.

Fig. 1 DMS depth profile.



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Derivation of K-rich ultramafic magmas from a peridotitic mantle source

THE genesis of a wide spectrum of basaltic magmas by partial melting of a peridotitic upper mantle with small amounts of H_2O as its only volatile component is well established¹⁻³, as is the role of CO_2 in magma genesis in the upper mantle^{4,5}. However, the origin of K-rich mafic to ultramafic magmas has not been satisfactorily explained by mechanisms involving partial melting of commonly accepted upper mantle sources. We report here experiments on an olivine ugandite from Bufumbira, south-west Uganda⁶, in conditions of H_2O and H_2O-CO_2 mixtures ($x_{CO_2} \sim 0.75$) up to 40 kbar, and at an $f_{O_2} \gg Ni-NiO$ buffer⁷. These are the first experiments reported on a lava of the ultramafic alkaline type with $K_2O > Na_2O$ using both H_2O and CO_2 as volatiles and showing that K-rich ultramafic magmas can be derived by partial melting of a 'normal' peridotitic mantle containing both H_2O and CO_2 . Olivine ugandite is believed to be the most primitive lava occurring in this K-rich province^{6,8-10} and may reasonably be considered as a model composition for K-rich ultramafic magmas generally.

Magmas with increasing degrees of undersaturation can be produced only by progressively decreased amounts of partial melting of a peridotitic upper mantle if H_2O is the only volatile component present³; olivine nephelinites or olivine basanites represent the most undersaturated types¹¹. Experiments up to 40 kbar on an olivine melilitite^{7,11,12} indicate that partial melting of an upper mantle source containing H_2O alone cannot produce such a magma, whereas partial melting of an upper mantle peridotite containing both H_2O and CO_2 ($x_{CO_2} \sim 0.75$) provides a satisfactory mechanism. Experiments on such magmas using both H_2O and CO_2 as volatile components have only involved compositions with $Na_2O \gg K_2O$. A high pressure study¹³ of a very K_2O -rich lava shows that such lavas cannot be produced by partial melting of a peridotitic mantle in which H_2O is the only volatile component.

Figure 1a shows the results of experiments in anhydrous, 5%, 15% and water-saturated conditions. The absence of orthopyroxene and garnet and the dominance of olivine and clinopyroxene at, or near, liquidus temperatures precludes the possibility of this magma being derived by partial melting of a peridotitic mantle source containing only H_2O . In contrast to studies of a composition richer in K_2O (ref. 13), phlogopite in the olivine ugandite does not occur in near-liquidus conditions (Fig. 1b), indicating that mechanisms of differentiation involving early fractionation of phlogopite¹³ are untenable.

Figure 1c shows phase relations with $x_{CO_2} \approx 0.75$ representing 27.5% weight CO_2 and 3.8% weight H_2O (equivalent to the 15% weight H_2O conditions of Fig. 1b). In these conditions, orthopyroxene, clinopyroxene and spinel are the near-liquidus

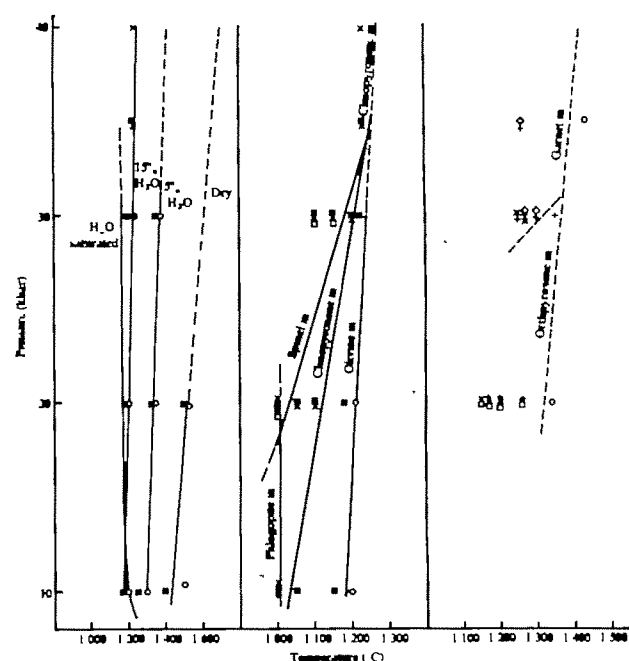


Fig. 1 P - T projection of olivine ugandite composition. a, Liquidus relations in anhydrous, 5% H_2O , 15% H_2O and H_2O -saturated conditions. b, Liquidus and subliquidus relations at 15% H_2O . c, Liquidus and subliquidus relations with H_2O and CO_2 at $x_{CO_2} \approx 0.75$ (mol %). O, Above liquidus; ■, olivine; x, clinopyroxene; +, orthopyroxene; ●, phlogopite; □, spinel; ◇, garnet.

phases at 20 kbar and orthopyroxene is the near-liquidus phase at 30 kbar, closely followed by garnet and clinopyroxene. Comparison of these results with those for an olivine melilitite^{7,11,12} indicates that the olivine ugandite could be produced by very small amounts of partial melting (<5%) of a peridotitic mantle source containing both H_2O and CO_2 .

Calculations using the microprobe analyses of the minerals crystallised in our experiments indicate that it is highly unlikely that mechanisms involving fractionation of garnet and/or orthopyroxene can develop a K-enrichment trend from parental magmas of olivine ugandite compositions. However, calculations using data on the minerals present in the runs done with 15% H_2O as the only volatile component (Fig. 1b) show that sequential fractionation of olivine, olivine + clinopyroxene, and olivine + clinopyroxene + phlogopite between about 20 kbar and 1,200 °C to about 10 kbar and 1,000 °C can produce successive liquids with compositions closely corresponding to the olivine ugandite-ugandite-olivine leucitite-leucitite series of the Bufumbira region⁶. Such liquids show an enrichment in K relative to Na (as $100 K/(K+Na)$) from 41 in the olivine ugandite to 55 in the leucitite, with a concomitant decrease in $100 Mg/(Mg+Fe^{2+})$ from 81 to 46.

On the basis of these experiments using olivine ugandite as a model parental composition, we propose that such magmas may be derived by small amounts of partial melting from a 'normal' (non K-enriched) peridotitic mantle source containing H_2O and CO_2 as essential constituents at ≥ 30 kbar and $\geq 1,350^\circ C$. In conditions of crustal warping due to rifting¹⁴, this magma may rise and its temperature decrease, producing a decrease in the solubility of CO_2 relative to H_2O (ref. 15) which suppresses crystallisation of garnet and orthopyroxene. At approximately 20 kbar and $1,200^\circ C$, olivine begins to fractionate, followed by olivine + clinopyroxene at lower pressures and temperatures (Fig. 1b). Residual liquids are depleted in MgO and enriched in TiO_2 , Al_2O_3 , CaO, Na_2O and K_2O . With further decrease in pressure and temperature, this residual liquid incorporates phlogopite which has previously crystallised from one of the liquids derived from the olivine ugandite or, less likely, from the olivine ugandite itself. This phlogopite may crystallise at ≈ 20 kbar (Fig. 1b) and rise by flotation to shallower depths equivalent to pressures of 10–20 kbar. With the addition of phlogopite and further fractionation of olivine and clinopyroxene, liquids substantially enriched in K_2O relative to Na_2O can be evolved at 10–20 kbar and $\geq 1,000^\circ C$. This mechanism of K-enrichment for lavas with $K_2O > Na_2O$ differs from those previously proposed^{6,8–10,16} in that the phlogopite is derived from the parental magma or its derivatives and is not a xenocrystic phase.

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Late Precambrian microfossils from Brioverian cherts and limestones of Brittany and Normandy, France

THE discovery of assemblages of unicellular, colonial and multiunit (endosporangium-like) algal microfossils in thin sections and in macerations of carbonaceous cherts and dolomitic limestones from the late Precambrian Brioverian 'Series' of northwestern France is reported here. These assemblages represent new links in the now rapidly expanding known record of Precambrian life. They are of interest because of (1) their similarity to microbiotas of comparable age reported from Canada and Alaska, a similarity suggesting that such assemblages could prove useful for global biostratigraphic correlation of late Precambrian strata; (2) their presence in partially metamorphosed sediments, an occurrence indicating that Precambrian organic-walled microfossils may not be as susceptible

to metamorphic alteration as has widely been assumed; (3) one of the assemblages is preserved in carbonates, a facies previously little studied by Precambrian micropalaeontologists.

Brioverian rocks are of demonstrably Precambrian age: they underlie lavas having a radiometric age of about 550 Myr (ref. 1); in Normandy, they unconformably underlie sediments containing a lower Cambrian fauna, and they are older than intrusive granites dated at about 580–620 Myr (ref. 2). The sequence is apparently substantially younger than 2,000 Myr, the approximate age of the underlying gneissic basement^{3,4}.

In Brittany and Normandy, Brioverian rocks can be subdivided into two age-groups: (1) a lower, metamorphosed sequence which in its upper portions contains metasediments (including black cherts locally known as 'phtanites') and which is older than 695 Myr, the age of plutonic massifs intrusive into the sequence^{2,3}; (2) a younger, unconformably overlying sequence which at its base locally contains volcanic material dated at about 640 Myr (ref. 4) and which is comprised largely of laminated shales and siltstones containing lenses of sandy dolomitic limestone. Three categories of microfossils (types I–III, below) were detected in lower Brioverian black cherts from the Pont–Mazet Quarry, near Quibou (between Coutances and St-Lô), Normandy, and from Le Pelikan (near Lamballe), Brittany; a fourth category (type IV) has been discovered in upper Brioverian dolomitic limestones from Vern-sur-Seiche (near Rennes), Brittany (Fig. 1).

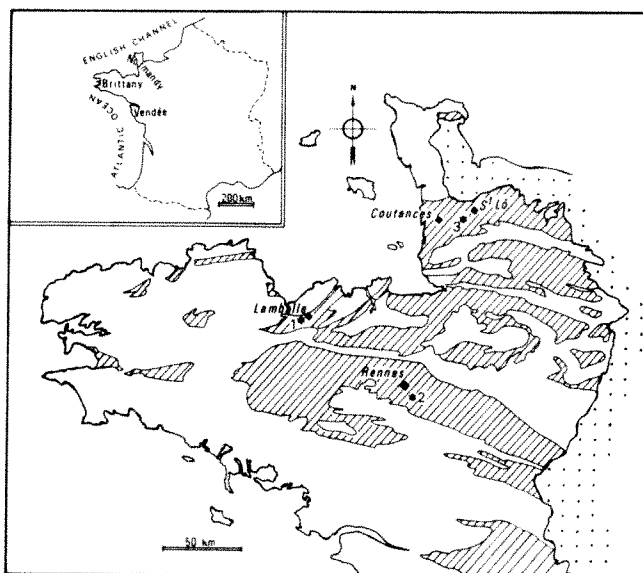


Fig. 1 Sketch map showing distribution of late Precambrian (cross-hatched) and Mesozoic–Cainozoic (stippled) sediments in Brittany and Normandy and locations of fossiliferous lower Brioverian black cherts (1, Le Pelikan, near Lamballe; 3, Pont–Mazet Quarry, near Quibou) and of upper Brioverian dolomitic limestones (2, Vern-sur-Seiche, near Rennes).

Like previously described microfossils from other fine-grained, carbonaceous Precambrian cherts^{5,6}, those of the lower Brioverian cherts have been preserved by siliceous permineralisation in a three-dimensional, structurally little altered condition (Fig. 2a–q). Organic matter comprising these fossils ranges in colour from light-grey to dark-grey or nearly black; like the particulate kerogen that is dispersed throughout these cherts, the carbonaceous microfossils were evidently heated, geochemically altered and 'coalified' during metamorphism of the sedimentary sequence. Microfossils detected in the upper Brioverian dolomitic limestones (Fig. 2r–v) are composed of light- to dark-brown organic matter, are permineralised in carbonate, rather than in silica, and in some cases seem to have been physically disrupted during compaction and diagenesis of the surrounding carbonate matrix (for example, Fig. 2v). These

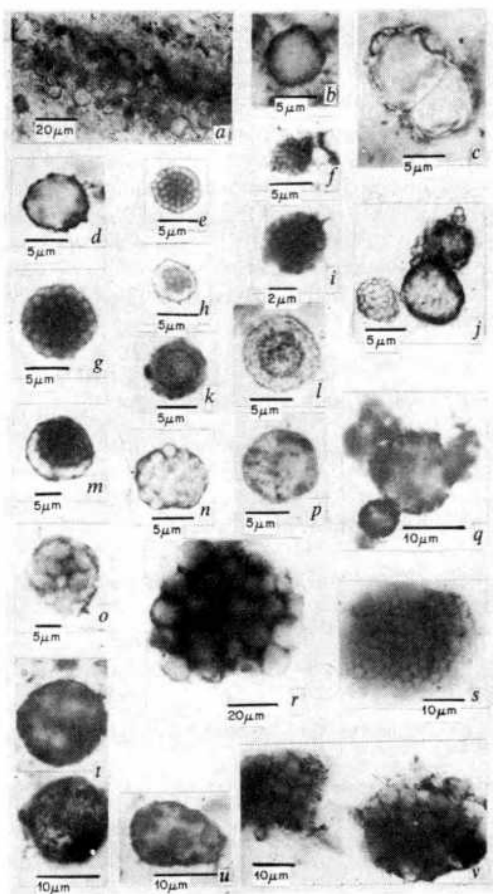


Fig. 2 Late Precambrian, organically preserved microfossils in thin sections (*a-c, f, i, j, l, p, r, s, v*) and in macerations (*d, e, g, h, k, m-o, q, t, u*) of lower Brioverian black cherts from Pont-Mazet, Normandy (*a-o*) and from Le Pelikan, Brittany (*p, q*), and of upper Brioverian dolomitic limestones from Vern-sur-Seiche, Brittany (*r-v*). Type I microfossils occur singly (*b, d, p, q*), in cell-pairs (*c*) or in irregular colonies (*a*); type II microfossils contain many spore-like coccoids (*e-m*); type III microfossils contain fewer, somewhat larger, spore-like bodies (*n, o*); type IV microfossils occur singly (*t, u*) or in globular colonies (*r, s, v*).

observations, together with studies of the textural relationships between these fossils and their encompassing mineral matrices and the fact that each of the four types of microfossils occurs abundantly (as demonstrated by studies both of acid-resistant macerations and of petrographic thin sections), establish that the Brioverian unicells and colonies are *bona fide* Precambrian fossils: they are indigenous to sediments of established Precambrian age and they were deposited syngenetically with Precambrian sedimentation.

Type I microfossils are spheroidal, thin-walled, unornamented unicells 2–15 μm in diameter (mean diameter = 5.6 μm , $n = 993$; Fig. 3*a*), that occur singly (Fig. 2*b, d, p*), in partially divided cell-pairs (Fig. 2*c*), or in mucilage-ensheathed, irregular colonies composed of a few to hundreds (Fig. 2*a*) of closely packed cells. Such cells comprise about 60% of microfossils detected in the lower Brioverian cherts.

Type II microfossils are spheroidal, noncolonial, multiunit cells, 2–12 μm in diameter (mean diameter = 4.9 μm , $n = 472$; Fig. 3*a*), that contain many spore-like, coccoidal bodies. These internal, closely packed coccoids, which in well-preserved specimens (for example, Fig. 2*e, g*) form three to four contiguous layers or 'shells' concentric about the centre of the spheroids, range in diameter from <0.5 to about 2 μm . A thin, unornamented cell wall can be discerned in those spheroids in which the spore-like coccoids only partially fill the enclosing cell (Fig. 2*h, m*), a condition resulting from degradation of the internal coccoids or, as evidenced by the common occurrence of

solitary, μm -sized, spore-like bodies in the cherts, from the release of such coccoids into the surrounding medium before preservation. Type II microfossils constitute nearly 40% of all forms detected in the lower Brioverian cherts.

Type III microfossils are noncolonial, multiunit, spheroidal cells 7–10 μm in diameter, that enclose two or three concentric 'shells' of closely packed, spore-like bodies (Fig. 2*n, o*). These bodies range in diameter from <2 to ~4 μm ; such cells thus contain fewer, but appreciably larger, coccoidal bodies than those contained in type II microfossils. Type III microfossils comprise only a small portion (<1%) of either of the two assemblages detected in the Brioverian cherts. Cells of this category, and of type II microfossils, are very similar in size to the type I microfossils described above (Fig. 3*a*). Indeed, except for the occurrence of coloniality (exhibited only by type I microfossils) and of distinctive, internal, spore-like bodies (characteristic of types II and III), members of the three categories of microfossils are virtually indistinguishable.

Thus, although additional morphotypes may also be represented, the lower Brioverian 'cherts' from the Pont-Mazet Quarry and Le Pelikan contain at least two morphologically distinct types of unicellular fossils: colonies and (at least in part) solitary type I microfossils; and type II (?) type III + (in part ?) solitary type I microfossils. These Brioverian assemblages bear only limited resemblance to microfloras previously described from late Precambrian cherts of western Europe⁷, for although simple 'type I-like' unicells (for example, *Paleocryptidium cayeuxi*) have been detected in Brioverian black cherts at Lamballe, Brittany⁹, and in late Proterozoic cherts of Bohemia¹⁰, they have not been found in association with the distinctive multiunit microfossils described here. In contrast, however, the Pont-Mazet and Le Pelikan assemblages seem strikingly similar to microfloras recently reported by Moorman from late Precambrian sediments of North America^{11,12}, microbiotas regarded by her as being composed solely of growth stages of a single taxon, *Sphaerocongregus variabilis*, a coccoid blue-green alga somewhat similar to the modern pleurocapsalean *Pleurocapsa fuliginosa*. According to Moorman¹¹, solitary or colonial unicells (as in our type I microfossils) represent vegetative growth stages of *S. variabilis* multiunit cells containing many small, spore-like bodies (as in our type II microfossils) are reproductive endospores, and multiunit cells that contain fewer, larger, coccoids (as in our type III microfossils) are sporangia containing 'nannocytes'. Fossils of the Pont-Mazet Quarry, Le Pelikan and North American assemblages seem virtually identical in morphology; thus, although the Brioverian material does not permit us to substantiate all phases of the "hypothetical growth cycle" proposed by Moorman for *S. variabilis*¹¹ (in particular, we find no evidence of the multiseriate,

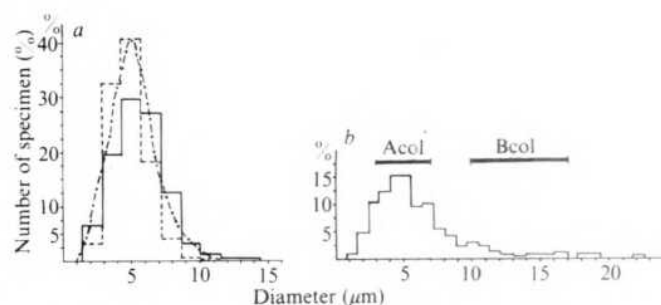


Fig. 3 *a*, Size distribution of colonial type I microfossils (---, $n = 260$, measured in thin sections), of solitary type I microfossils (—, $n = 733$, measured in macerations), and of type II microfossils (---, $n = 472$, measured in macerations) from lower Brioverian black cherts of Normandy and Brittany. *b*, Size distribution of type IV microfossils from upper Brioverian limestones of Vern-sur-Seiche, Brittany; histogram shows sizes of solitary cells measured in macerations ($n = 386$); Acol ($n = 29$) and Bcol ($n = 23$) show size ranges for cells in two well-preserved colonies, measured in thin sections.

vegetative, filament-like structures she depicts), these newly discovered assemblages support several of her interpretations. Specifically, it seems increasingly plausible (1) that the multiunit cells may represent reproductive stages, and that the solitary and colonial cells may be vegetative stages of a single taxon; (2) that this taxon is probably of blue-green algal affinity (although its referral to the Pleurocapsales remains in question, as endosporeulation also occurs among chroococcalean and chamaesiphonacean cyanophytes); and (3) that *S. variabilis* was chiefly planktonic, rather than benthonic, in habit (an interpretation supported in both the North American and French material by, among other lines of evidence, the more-or-less random distribution of the fossils within the sediments). In addition, the Brioverian specimens of *Sphaerocongregus*, in which the cells are preserved as organic residues rather than as 'pyritic replacements'¹², confirm that the multiunit cells are authentic microfossils rather than the nonbiological pyrite framboids to which they bear superficial resemblance^{13,14}.

In North America, *S. variabilis* has been detected in shales of the 850–570 Myr-old Hector Formation of the Windermere Supergroup of southwestern Alberta, Canada¹¹, and in carbonaceous shales and mudstones of approximately the same age from the Tindir Group of east-central Alaska, USA¹². Multiunit microfossils similar to the types II and III microfossils described here have also been reported from sediments of the Soviet Union (small-celled species of *Symplastosphaeridium* Timofeev = *Microconcentrica* Naumova) in which they reportedly range in age from the mid-part of the Middle Riphean (about 1,100 Myr in age) into the Late Cambrian¹⁵. Based on these known distributions, and in view of stratigraphical and geochronological data available for the Brioverian sequence, it seems reasonable to suggest that the fossiliferous lower Brioverian phanites at Pont-Mazet and Le Pelikan are probably Middle to Late Riphean (1,100–695 Myr) in age.

Type IV microfossils (Fig. 2*r–v*), detected in upper Brioverian (Vendian age) sandy dolomitic limestones from Vern-sur-Seiche (Fig. 1), are spheroidal, relatively thick-walled (Fig. 2*r, t*), unornamented unicells, 1–20 µm in diameter (mean diameter = 5.9 µm, *n* = 386; Fig. 3*b*), occurring singly (Fig. 2*t, u*) or in globular colonies composed of a few to many cells (Fig. 2*r, s, v*). Colonies range from <30 to >70 µm in diameter and have a measured abundance of about 50 colonies per cm³ of rock; they most commonly occur within (and tend to be orientated parallel to) fine, organic, bedding-plane laminations, a distribution suggesting that they were predominantly benthonic in habit. In size, shape and colonial organisation, type IV fossils bear considerable resemblance to modern, coccoid (for example, chroococcalean) blue-green algae, and the broad size range exhibited by the total assemblage, in comparison with the rather uniform size of member of single colonies (Fig. 2*r, s*; Fig. 3*b*), indicates that several cyanophytic taxa are probably represented. Fossils of this size and organisation have been reported from many Precambrian deposits⁵; the Vern-sur-Seiche assemblage, however, is unusual in that only one¹⁶ other of the nearly four dozen permineralised microbiotas now known from the Precambrian^{5,6} has been discovered in carbonates, the remaining permineralised assemblages occurring exclusively in bedded cherts or in cherty portions of algal stromatolites.

In addition to representing new links in the now rapidly expanding knowledge of Precambrian life, the three microfossil assemblages described here are of interest for the following reasons: (1) two of these assemblages, those occurring in the lower Brioverian phanites, are similar in composition to microfossils of apparently comparable age in North America; taken with other data⁵, these similarities suggest that such assemblages may ultimately provide a reliable basis for global biostratigraphic correlation of Precambrian strata. (2) The occurrence of structurally preserved microorganisms in partially metamorphosed Brioverian cherts seems to indicate that Precambrian organic-walled microfossils may not be as susceptible to metamorphic alteration as has widely been assumed; there is thus good reason for continued palaeobiological and biostratigraphical studies of such facies, especially in western France where many cherts of similar metamorphic grade but of uncertain geological age occur. (3) The occurrence of permineralised, well-preserved microfossils in upper Brioverian limestones suggests that in addition to shales and cherty facies, carbonates represent a productive, but as yet largely untapped, source of new evidence regarding the diversity, evolution and biostratigraphical usefulness of the Precambrian biota.

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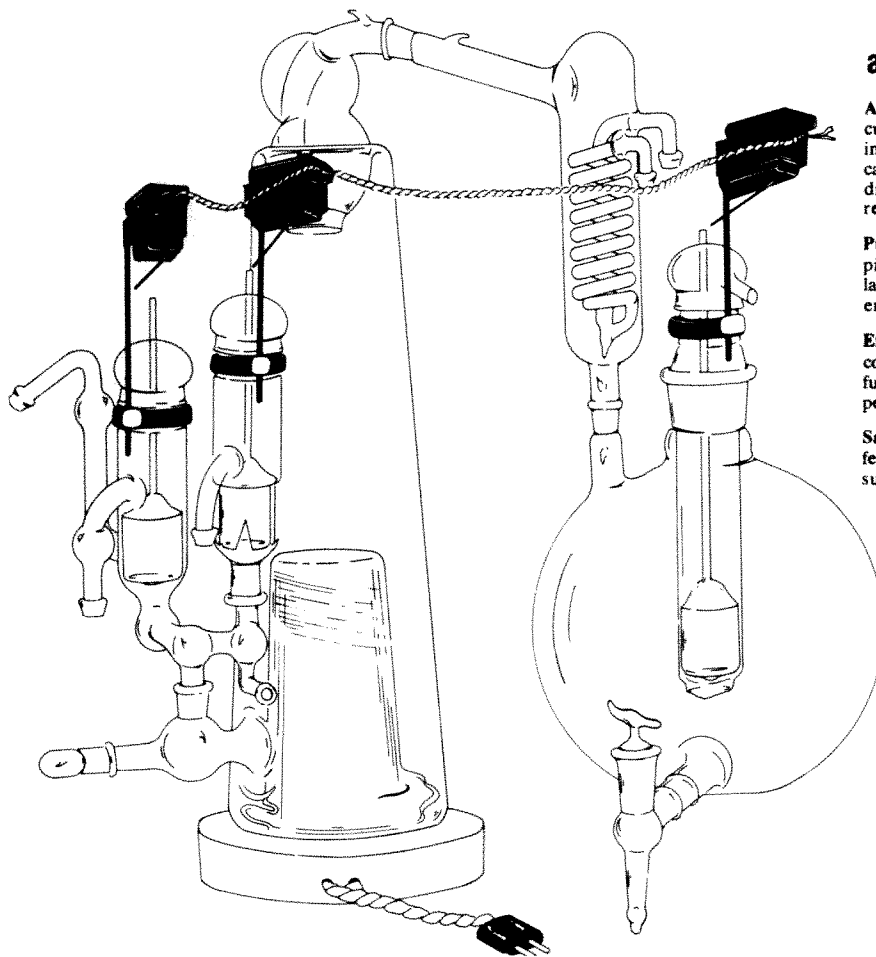
Influence of a predator on the optimal foraging behaviour of sticklebacks (*Gasterosteus aculeatus* L.)

ACCORDING to the principle of natural selection, each individual animal is assumed to maximise its inclusive fitness¹. Thus, observed behaviour patterns should result from optimisation processes involving costs and benefits measured in a currency of fitness^{2,3}. Certain foraging strategies have been shown to maximise energy intake per unit time^{3–6}. Maximisation of the rate of energy intake, however, is an optimal strategy only if feeding behaviour does not conflict with other needs, such as the detection of predators. If the foraging animal runs a high risk of being preyed upon, the optimal strategy may be a compromise of both needs. A predator's influence on optimal foraging has been dealt with, so far, only in a hypothetical manner^{3,5}. We have investigated this problem experimentally using three-spined sticklebacks (*Gasterosteus aculeatus*) attacking a swarm of water fleas (*Daphnia magna*). We report here that after exposure to a model of an avian predator the sticklebacks' foraging behaviour changes such that they attack swarm regions of lower density which provide a lower feeding rate but should increase their ability to detect an approaching predator. This is predicted by a model using Pontryagin's principle of maximisation.

Sticklebacks first attacked swarm regions of high prey density, but with decreasing attack readiness (for example, induced by decreasing hunger) showed a preference for less dense regions, directing their last attacks at stragglers⁷. A high swarm density

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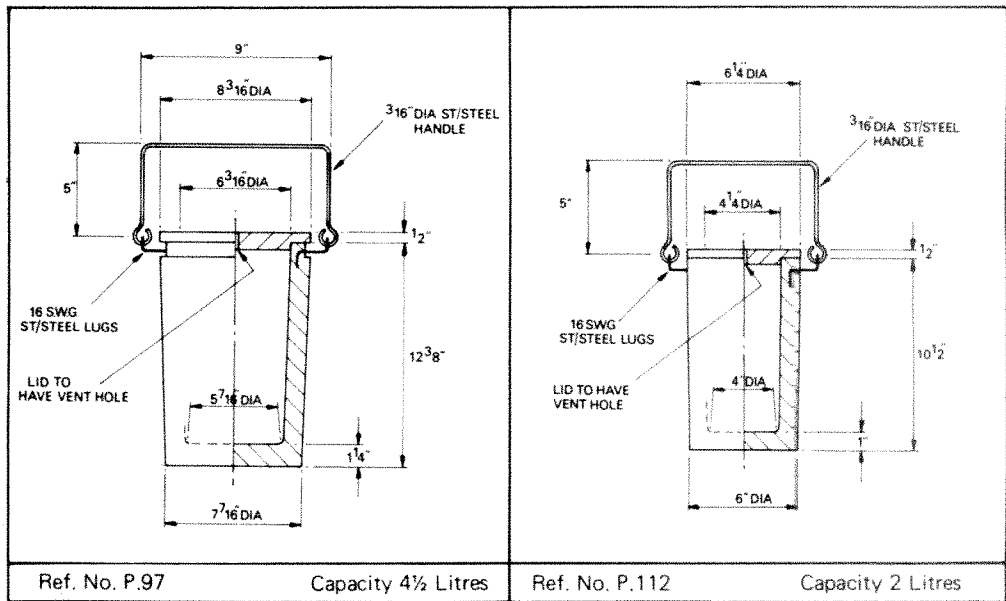
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enables the predator to achieve a high feeding rate because of short inter-prey distances. However, at high prey densities the predator has increased difficulty in aiming at individual prey targets^{8,9} ('confusion effect'). Thus it seems likely that feeding in high swarm densities reduces the stickleback's ability to pay attention to other visual stimuli, for example, an approaching predator.

We have developed a model representing the situation of a stickleback confronted with a swarm of water fleas. At any time t its costs in terms of fitness units, result from its hunger state $s(t)$ and confusion while feeding at a capture rate $c(t)$ in a density $d(t)$. The dependence of fitness on hunger state is assumed to be $\alpha s^2(t)$, due to an increased risk of starvation², the costs resulting from confusion are assumed to be $\beta d(t)c^2(t)$, due to a higher vulnerability in the face of predators (greek symbols are used as constants of proportionality to enable adjustment of considered influences). These costs will rise with increasing feeding rate and/or density (our unpublished results). During a given time interval $(0, T)$ the stickleback should minimise its total costs². In other words it will minimise the integral value

$$\int_0^T \alpha s^2(t) + \beta d(t)c^2(t) dt \quad (1)$$

Because the decrease in hunger is proportional to the capture rate we have

$$\frac{ds}{dt} = -\gamma c(t) \quad (2)$$

Capture rate $c(t)$ and density $d(t)$ cannot vary independently, because a high capture rate is impossible at a low prey density. On the other hand $c(t)$ is limited by the minimum time taken to swallow an item of prey; that is

$$(a) \delta c(t) \leq d(t) \quad \text{and} \quad (b) 0 \leq c(t) \leq c_{\max} \quad (3)$$

Therefore the stickleback is able to vary $d(t)$ and $c(t)$ freely in an area under a linear curve reaching a plateau. The predictions deduced from the model will remain true if a dependency similar to Holling's disk equation is assumed. In order to choose the combination of $d(t)$ and $c(t)$ with lowest costs, equality will hold in equation (3a). According to Pontryagin's maximum principle, (1) can be minimised by maximising the hamiltonian¹⁰

$$H(c, t) = -[\alpha s^2(t) + \beta \delta c^3(t) + \gamma c(t)p(t)] \quad (4)$$

with unknown adjoint variable $p(t)$. This gives

$$c(t) = \begin{cases} c_{\max} & p(t) < -\frac{3\beta\delta c_{\max}^2}{\gamma} \\ \sqrt{\frac{\gamma p(t)}{3\beta\delta}} & \text{otherwise;} \\ 0 & p(t) > 0 \end{cases} \quad (5)$$

$d(t) = \delta c(t)$ always.

By solving the system of first order differential equations:

$$\frac{ds}{dt} = -\gamma c(t), \quad \frac{dp}{dt} = 2\alpha s(t) \quad (6)$$

the optimal courses of $s(t)$, and therefore $c(t)$ and $d(t)$, can be calculated. This model leads to the following predictions. First, the stickleback has to start in a high density with a high feeding rate; with decreasing hunger, a lower feeding rate should be achieved with less confusion at a lower prey density (our unpublished results and Fig. 1). Second, if the stickleback's costs of confusion are raised because of a predator presence, a lower capture rate within a lower density will be optimal at the start (Fig. 1). Higher capture rates for some time provide a lower

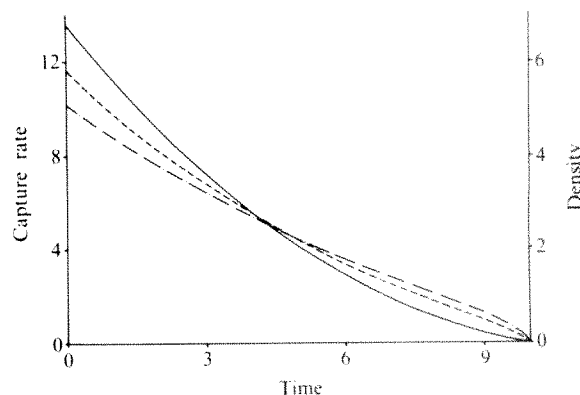


Fig. 1 Optimal courses of capture rates and densities in time (arbitrary units) for different risks of predation. For clarity the same set of curves indicates both the appropriate capture rates, when read with the left scale, and simultaneously the densities, when read with the scale on the right. The more elevated the risk of predation the lower should be the stickleback's initial capture rate and its aimed density. —, low risk; — — —, medium risk; — · —, high risk.

hunger state after this time. Thus sticklebacks initially feeding faster, will have lower capture rates in lower densities afterwards.

Thus in the presence of a predator a hungry stickleback which would normally attack the most dense region of a swarm with a high feeding rate, has to divide its attention between prey and predator and would be expected especially at the start to slow its feeding rate and to attack a region inducing less confusion, that is, a swarm region of a lower density. These qualitative predictions have been tested by the following experiments.

A tank (25.5 × 12.5 × 17 cm) was divided into halves by a partition which included a sliding door. In front of the back wall of one half of the tank there was a transparent plexiglass cell containing the prey, 9 cm from the sliding door. This cell was divided into seven adjacent compartments, each with a base of 1 × 1 cm. These contained 0, 2, 0, 40, 20, 2, and 0 water fleas of approximately equal size (water level 1.5 cm). To simulate the presence of a stickleback¹¹ predator a black silhouette of a European kingfisher (*Alcedo atthis*, 16 cm beak to tail length) suspended on two nylon threads was made to glide over the other half of the tank (start compartment) at a mean distance of 41 cm and at an angle of 50° subtended horizontally. To ensure that the bird stood out from the 'sky' there was a large white canvas sheet 2 m above the tank illuminated from above by a 500 W bulb. Two alternating experiments (with and without predator) were conducted.

Before each trial three water fleas and then a stickleback which had last fed on the previous day were transferred to the start compartment. In the first experiment after the *Daphnia* had been eaten the silhouette was made to glide over the tank and then the sliding door was opened. In the second experiment the bird was not shown. The initial meal of three *Daphnia* ensured that the stickleback reacted to the cell. All fish passed through the door within five minutes and began biting at the prey-containing cell. Attacks on the prey cell were recorded until there was no bite for at least one min. Each fish was used only once. Mean fish length did not differ significantly between experiments.

The sticklebacks in the presence of the predator (disturbed) hesitated longer between door and first bite (median 10.2 s; quart. 7.1 and 18.8) than those in its absence (undisturbed) (median 6.7 s; quart. 5.2 and 7.7; $P < 0.01$, Mann-Whitney U -test, 2-tailed). The undisturbed fish initially attacked the most dense region whereas the disturbed sticklebacks preferentially the least dense region, that is the stragglers (Fig. 2). In subsequent attacks the disturbed fish continued to prefer the two least dense regions and the stragglers in

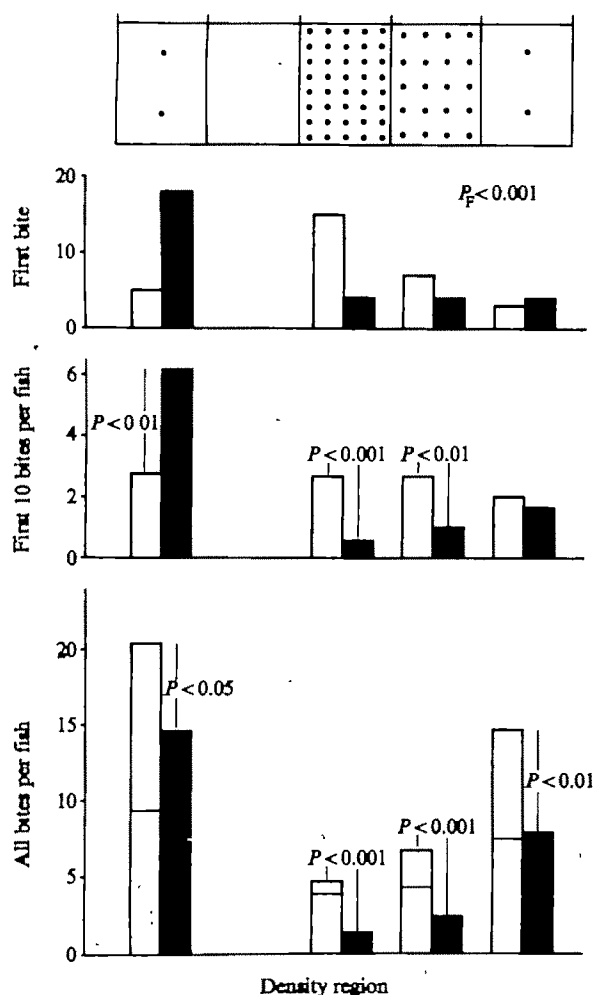


Fig. 2 Attacks in relation to swarm density in the presence (black columns) and absence of the predator model (white columns). Parts of columns below bar represent the first half of total number of attacks. $n = 60$; P after Mann-Whitney U -test, 2-tailed; P_F after Freeman-Halton test.

particular. With decreasing attack readiness the undisturbed sticklebacks changed their initial preference for dense regions to a preference for the least dense regions (Fig. 2). During the first ten bites, each region was attacked nearly equally often, during the first half of the total reaction, there was a slight preference for the less dense regions, while the stragglers were strongly preferred in the second half. Overall, the disturbed fish bit less often (26.0 ± 16.8) than the undisturbed ones (49.7 ± 23.1 , $P < 0.0001$, Mann-Whitney U -test, 2-tailed).

The presence of any predator, for example the kingfisher, should distract the fish's attention when detected. Indeed sticklebacks showed a marked fright response to a dark object about the size of a bird moving above them^{12,13}. It seems improbable that the change in the sticklebacks' preference is an epiphenomenon of a more general adaptation to shock, because electric shock increases the sticklebacks' feeding intensity¹⁴, whereas our disturbed fish decrease their feeding intensity. Also in starlings, surveillance diminished their feeding activity¹⁵. There is good reason to believe that a decrease in motivation to bite against the prey cell acts in a similar way on chosen densities and capture rates as a decrease in hunger (our unpublished results). Thus, as proposed by the model, the changed foraging behaviour is an optimal strategy supplying both needs, namely to pay attention to a predator and with the remaining attention to maximise energy intake by attacking a less confusing swarm density.

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Effect of reduced litter size on the suckling behaviour of developing rats

DURING the past 20 years considerable attention has been drawn to the effects of altered litter size on the growth and development of various mammals. Studies with rodents have shown that increasing litter size permanently reduces body growth by attenuating the rate of cell division and the final number of cells per organ¹⁻⁵. Conversely, reduction of litter size enhances growth and development^{1,6-9}. Altering litter size has also been shown to effect adult behaviour; by elevating emotional reactivity^{8,12}, and reducing learning ability¹³ in pups raised in large litters, and by reducing emotionality in pups raised in small litters¹⁰⁻¹². It is not known, however, how quickly behavioural differences may result from alterations in litter size. Moreover, it is unclear whether such behavioural effects are a consequence of nutritional changes or of alterations in the pups' developmental experiences. For example, behavioural differences may be due to alterations in maternal behaviour, as rats nursing small litters spend more time in the nest with these pups, and more time handling and manipulating them^{14,15}. Additionally, varying litter size probably alters within litter competition for the nipples, and this may effect behavioural development as well. Utilising recently developed techniques for the measurement of suckling¹⁶⁻¹⁸ and activity¹⁹ we attempted to discover whether altering litter size directly affects the behaviour of pre-weanling rats. We now report that reducing litter size has the surprising effect of reducing both the willingness of pups to suckle and the vigour of their search behaviour on the mother. Moreover, these effects seem more directly linked to alterations in the litter experience than to nutritional differences.

Sprague-Dawley rats were mated and bred in our own colony. Each experimental comparison was made between two litters of pups born within 3 h of each other. In half the replications the pups were randomised between the mothers and culled to produce a normal size litter ($\bar{x} = 12$, range 8-16) and a small litter of three pups. In the other replications the same litter sizes were used but each pup remained with its natural mother. Culling took place on the day of birth, which was considered day

0. Altogether 36 pups raised in litters of three, and 144 pups raised in 12 natural size litters were studied. Half of the litters were tested on day 5, and the remainder on day 10.

Five hours before the start of testing half the pups from the large litter and randomly either 1 or 2 pups from the small litter were removed and placed in an incubator. These pups constituted the deprived groups. Following deprivation each mother was anaesthetised (Chloropent, 2 ml per kg body weight, intraperitoneally, Fort Dodge Laboratories) and laid supine in a clear plexiglas trough. Both deprived and nondeprived pups were placed individually next to the mother and tested for nipple attachment^{17,18}. Numbers of pups attaching and latency scores were recorded. Each pup was tested for 5 min. Pups were tested both on their own mother and the paired complementary mother.

In addition to this suckling test, a second procedure was used¹⁶. In this test each pup was independently held and gently positioned so that its snout came into direct contact with a presuckled nipple. Attachment latencies were recorded, and a 1 min ceiling was used. Previous research has shown that these are both sensitive tests for the appetitive components of suckling, though the former test (pups on mother) requires that the pups locate the nipples while the latter (pups on nipple) does not¹⁸.

Maternally-directed activity was measured using a digital readout vibrational activity monitor¹⁹ modified to record activity only when the pup was in the immediate vicinity (3 cm) of the mother. Each pup was placed on the sensitive surface with its snout in direct contact with its anaesthetised mother. Activity was recorded for 15 s, which was sufficient time for an accurate measurement of maternally-directed exploration, but was too brief to permit the pups to attach to a nipple.

By 10 days of age serious deficits in suckling behaviour were noted in pups raised in small litters (see Fig. 1). Per cent attachment fell from 100% to 50% (deprived) and from 92% to 0% (nondeprived) on the pups on mother test ($z = 4.49$, $P < 0.001$; $z = 5.42$, $P < 0.001$. Test for the significance of the difference between two proportions²⁰). Testing the pups from the small litters on the mother raising the larger litters had absolutely no restorative effects on suckling. This indicates that the deficit is primary to the pups and is not simply due to some inadequacy of their mother as a target stimulus. Furthermore, the deficits were fully apparent when the pups were placed in contact with highly salient nipples; falling from 100% to 30% (deprived $n = 10$), and from 94% to 25% (nondeprived $n = 8$) respectively ($z = 5.43$, $P < 0.001$; $z = 4.61$, $P < 0.001$).

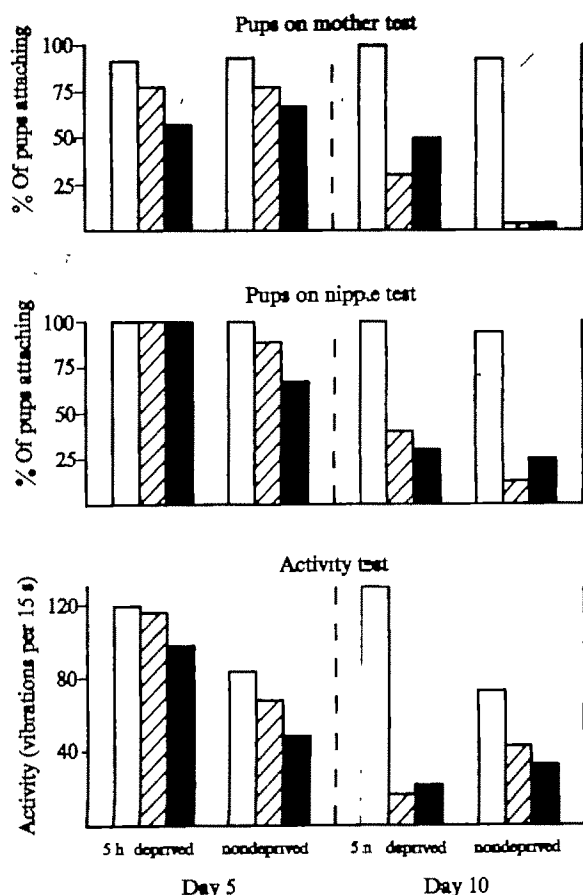
These deficits in nipple attachment were in part mirrored in the activity scores. Normally pups of these ages show an immediate response to anaesthetised mothers, and begin searching the mother's ventrum for nipples. Raising pups in small litters attenuated this behaviour ($F(1,40) = 11.03$, $P < 0.005$; main effect of litter size), though the effect was age dependent, showing up only at day 10 ($F(1,40) = 6.56$, $P < 0.025$; interaction of age $\times$ litter size, day 5: $t(22) = 0.39$, not significant, day 10: $t(22) = 5.80$, $P < 0.001$), and more specifically within the normally very vigorous deprived group ($F(1,40) = 4.24$, $P < 0.05$ interaction of age $\times$ litter size $\times$ deprivation; day 10 deprived: $t(10) = 13.73$, $P < 0.001$, day 10 nondeprived: $t(10) = 2.11$, $P < 0.10$). This observation highlights the fact that the failure of deprived 10-day-old pups to locate and suckle in these test protocols was not just due to an inability to find the nipple (as happens to normal pups on washed mothers²¹) but stems from a general failure to initiate any sort of active search process.

The suckling behaviour of 5-day-old pups however, was less markedly affected by alterations in litter size. Both deprived and nondeprived pups raised in litters of three attached in fewer numbers than normally reared pups when they were required to forage for the nipples of the mothers with which they were reared (deprived: $z = 2.69$, $P < 0.01$; nondeprived: $z = 2.28$, $P < 0.025$). Nondeprived pups also attached in fewer numbers when held in contact with these nipples ($z = 3.58$, $P < 0.001$), although deprived pups attached as readily as controls. Overall,

these deficits in suckling behaviour were less pronounced than those seen on day 10. Moreover, these early deficits were partially ameliorated by testing the pups raised in small litters on mothers raising larger litters, and in most instances significant litter size effects were no longer seen (pups on mother: deprived, $z = 1.21$, not significant; nondeprived $z = 1.49$, not significant; pups on nipple: nondeprived, $z = 2.07$, $P < 0.05$). In short, the suckling behaviour of 5-day-old pups raised in litters of three is slightly abnormal and exacerbated by the apparently poor mammary development of mothers raising these small litters.

We have demonstrated that raising pups in litters of three markedly affects their pre-weaning suckling behaviour. This finding is of particular importance because we are able to detect the litter size effect at such an early age. However, it raises the interesting question of what factors, nutritive or experiential, create these differences in behaviour. Are the deficiencies in nipple attachment of 10-day-old pups raised in small litters due directly to their elevated body weights (29.5% greater than controls) or to certain experiential differences such as reduced competition in the smaller litter? Fortunately, the general facilitatory effect of reduced litter size on body weight and growth was not absolute, but was relative to the body weight of the mother. Thus pups raised in a litter of three with a small mother frequently weighed no more than siblings raised in a litter of 12 with a much heavier mother. This allowed us to separate experiential effects from nutritive effects. In doing so it became clear that litter size *per se* and not body weight was the crucial factor. Pups reared in litters of three with light mothers (who obtain only normal weight levels) clearly manifest the same deficits in nipple attachment as much heavier pups reared in

Fig. 1 Effects of reduced litter size on suckling (pups on mother and pups on nipple test) and maternally-directed activity. Litters were culled and pups cross-fostered on the day of birth to produce both natural ($\bar{x} = 12$ pups) and small (3-pup) litters. Pups were tested at either 5 or 10 days of age. Open columns, litters of 12 tested on mother raising litters of 12; cross-hatched columns, litters of 3 tested on mothers raising litters of 12; solid columns, litters of 3 tested on mothers raising litters of 3.



litters of three with large mothers. The correlations between litter size and attachment latency for both test protocols were very high even when body weight was excluded from the analysis^{22,23} ($r = -0.935$ and -0.732 pups on mother and pups on nipple test respectively, all P -values < 0.001). On the other hand, the correlations between body weight and attachment latencies were insignificant when litter size was excluded ($r = -0.234$ and 0.114 respectively, all P values not significant). This result strongly suggests that the litter size manipulation was not affecting suckling simply through its modulation of food availability and body weight. Rather, some experiential aspect of the situation seems to be influencing the pups behaviour. One possibility is that the reduced level of competition in the small litters was not adequate to maintain performance on these suckling tasks, while an alternative possibility is that pups from small litters are accustomed to, and require, a great degree of maternal stimulation before suckling and fail to suckle anaesthetised mothers who do not provide this stimulation.

Overall, it is apparent that raising pups in litters of three attenuates both the appetitive component of suckling, and the vigour with which these pups search their anaesthetised mothers. These differences were small at day 5 and to a large extent reflected differences between the mothers, more than differences between the pups. By day 10 however, deficits in suckling behaviour were marked, and were primary to the pups. Finally, although rats raised in small litters usually weigh more than siblings raised in larger litters, this increase in body weight was not a necessary condition for the disruption in suckling behaviour. It may seem paradoxical for 10-day-old pups from small litters to weigh more than siblings raised in large litters given their apparent hypomotivational state. However, it must be pointed out that the mother's lactational capacity is clearly the rate limiting factor for weight gain in pre-visual pups raised in large litters^{24,25}. Thus, although these pups may be more vigorous in their suckling behaviour, their body weights are in fact limited by restricted food availability.

This study demonstrates that early experience may have significant effects on the ontogeny of ingestive behaviour. What precisely these experiences are, and whether their effects extend beyond the suckling period, remains to be determined. However, this study clearly indicates that 'nutritional' studies relying on litter size manipulations may be producing effects which are not in fact due primarily to changes in nutrition.

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Thermal acclimation in temperate lizards

THERMAL acclimation has been studied most intensively in aquatic ectotherms¹ in which body temperature closely approximates water temperature, which changes slowly with season. As aquatic ectotherms must therefore be active over a range of slowly changing body temperatures, acclimation usually functions as a homeostatic mechanism; following a temperature change in the environment, the organism's metabolic rate is adjusted so that it is returned to near its original level². Some terrestrial animals, particularly reptiles which thermoregulate behaviourally, are subject to considerable daily fluctuations in body temperature³. This difference in the thermal ecology of aquatic ectotherms and terrestrial reptiles, which may cause a difference in the acclimatory responses of the two groups, has not been considered in the work on thermal acclimation in reptiles⁴⁻⁶. Consequently, this work is of little use in predicting the acclimatory responses of reptiles to seasonal temperature changes in the field. We present here evidence that lizards allowed to thermoregulate behaviourally acclimate only at low body temperatures and that the common pattern of acclimation differs from that of aquatic organisms in being determined by requirements of low temperature energy conservation rather than metabolic homeostasis.

Males of four lizard species were used, two from a warm temperature climate (*Podarcis hispanica* and *Psammotriton hispanicus*) and two from a cool temperate climate (*Lacerta vivipara* and *Anguis fragilis*). The experimental design was similar for all species. Lizards were housed in vivaria in a constant temperature room with the temperature adjusted to the mean night time temperature of the season under study. Heat lamps in the vivaria were turned on daily for a period corresponding to the mean photoperiod of the season (Table 1). When allowed access to these lamps, the lizards maintained a relatively constant body temperature which was considerably higher than, and independent of, shade air temperature. At night, with the heat lamps off, the lizards' temperatures fell quickly to ambient. Thus, under these experimental conditions, the lizards were subjected to a fluctuating daily temperature

Table 1 Photoperiod and night time temperature regimes used in the seasonal acclimation study

	Climatic type			
	Warm temperate		Cool temperate*	
	Night time temperature (°C)	Photoperiod (light:dark) (h)	Night time temperature (°C)	Photoperiod (light:dark) (h)
Spring	10	13:11	5	13.75:10.25
Summer	20	14:10	15	15.50:8.50
Autumn	15	11:13	10	10.75:13.25
Winter	5	10:14	—	—

* Cool temperate lizards were studied only in spring, summer and autumn because they are in hibernation, and therefore not exposed to a fluctuating temperature regime, in winter.

regime similar to that experienced under natural conditions. All animals were maintained in this way for at least a month before measurements of metabolic rate were made. Metabolic rate was measured as the rate of oxygen consumption of lizards in small constant pressure respirometers immersed in a constant temperature water bath. All measurements were made on quiescent, post-absorptive animals and therefore approximate to standard metabolic rate. Oxygen consumption rates were measured at 5 degree intervals from 5 °C (the lowest temperature experienced by any animal under the experimental regime) to 30 or 35 °C (the upper body temperatures attained when the heat lamps were on⁷).

For each species, seasonal acclimation had little or no effect on rates of oxygen consumption at the higher temperatures, although significant differences between seasonal groups occur at the lower temperatures for all species (Fig. 1). In other words, differences in metabolic rate do not generally occur when oxygen consumption is measured at temperatures experienced by all groups every day; it is only at temperatures experienced by some seasonal groups but not by others (the lower temperatures) that differences in metabolic rate occur. These seasonal differences in low temperature metabolic rate can be interpreted as mechanisms for energy conservation. In *Podarcis hispanica*

and *Psammodromus hispanicus* (Fig. 1a, b) low temperature metabolic rates are lowest in winter and spring. In the region where these lizards originate, it is at these seasons that there are the longest periods of adverse weather when the lizards cannot come out to bask and feed. In *Lacerta vivipara* and *Anguis fragilis* (Fig. 1c, d) low temperature respiration is less in autumn than in spring or summer; this is possibly a mechanism for reducing low temperature energy expenditure in cool temperate animals entering or about to enter hibernation.

As many reptiles utilise solar radiation to maintain high, relatively constant body temperatures^{8,9}, it is not surprising that little or no thermal acclimation occurs at high temperatures because such body temperatures can be attained each day that the sun shines. To the animal, the main thermal difference between the seasons is the temperature to which its body drops at night, and it is at these low temperatures that seasonal differences in metabolic rate (acclimation) occur. The animal is incapable of feeding at these low body temperatures and it is therefore energetically efficient to effect some measure of energy conservation when the animal has to spend long periods at low temperatures. Thus the common pattern of acclimation in temperate reptiles is determined by considerations of low temperature energy conservation. Some other terrestrial ectotherms also thermoregulate behaviourally¹⁰⁻¹² and it is possible that this pattern of acclimation is widespread in terrestrial animals.

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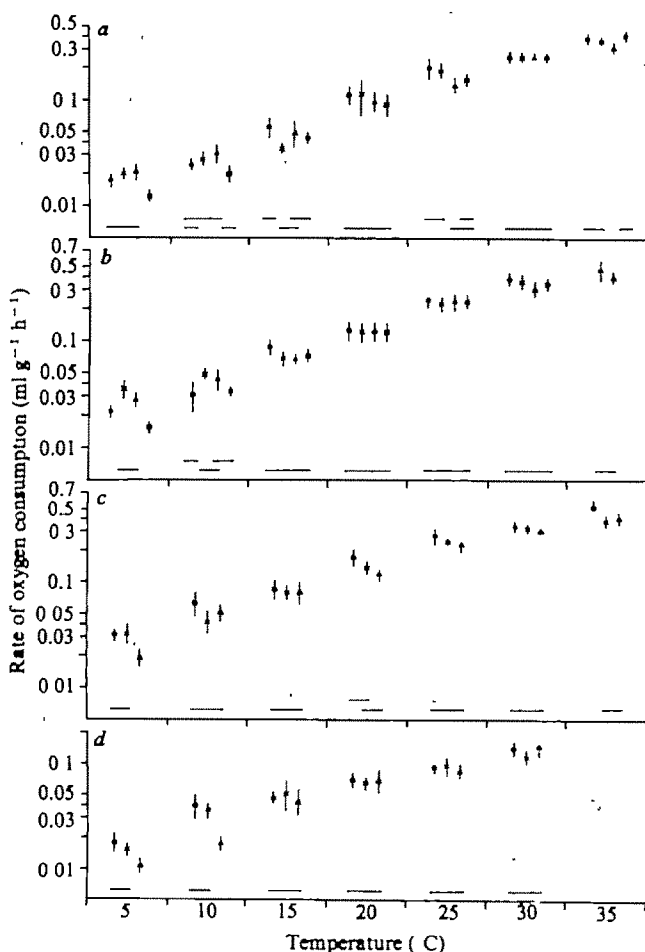
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Fig. 1 Rates of oxygen consumption at different temperatures for the different seasonal acclimation groups for males of the four lizard species a, *Podarcis hispanica*; b, *Psammodromus hispanicus*; c, *Lacerta vivipara*; d, *Anguis fragilis*. ○, Mean rates of oxygen consumption for spring acclimation groups; ×, mean rates of oxygen consumption for summer acclimation groups; △, mean rates of oxygen consumption for autumn acclimation groups; □, mean rates of oxygen consumption for winter acclimation groups. The vertical lines above and below each mean are 95% confidence limits of that mean. At each temperature, means not underscored by a common line or a line at the same level are significantly different from each other (analysed by one-factor analysis of variance and Duncan's 5% new multiple range test).



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Feeding, blood glucose and plasma insulin of mice at dusk

MOST omnivorous species exhibit a circadian rhythm of food intake. During one part of the day food is consumed at a rate in excess of immediate metabolic needs, and the surplus is stored to support metabolism for the remainder. Le Magnen and Devos¹, for example, have shown a predominance of lipogenesis in the night-time feeding period of the rat, and pronounced lipolysis during the day, when little food is consumed. A variety of metabolic adaptations exists to match this discontinuous pattern of food intake with relatively constant metabolic use of nutrient², but the question remains whether the hyperphagia at one time of day produces these changes or is a consequence of them. Le Magnen suggests the latter³, at least in the case of increased night-time meal frequency, and in models of feeding, such as that of Toates and Booth⁴, an increased rate of metabolic

clearance of food at night is a considerable factor in promoting increased food intake. On the other hand, eating at a high rate clearly will promote metabolic changes, as bunching of the normal daily intake into a very short period will lead to increased weight gain⁵. A compromise possibility is that a greatly increased rate of feeding at dusk rapidly promotes metabolic changes subsequently favouring a high rate of feeding in the ways suggested by previous authors. The results reported here suggest that such a mechanism may operate in the feeding of mice, in that a great increase in feeding rate around the onset of darkness produces changes in carbohydrate metabolism which may both favour storage of energy and tend to increase subsequent food intake.

All experiments used individually caged female C57 mice, ~100 d old at the start of the experiments, and kept for their entire lives in a temperature-controlled room ($23 \pm 1^\circ\text{C}$), on a constant 12-h light, 12-h dark cycle (lights off 13.00). Unless stated otherwise, standard laboratory food (Spratts rodent breeding diet) and water were available *ad libitum*. Food intake was measured where necessary by regularly weighing feeder inserts in the cages⁶. Blood samples were withdrawn from a tail vein within 2 min of removal from home cage (this technique yields assay results indistinguishable from blood obtained by immediate decapitation). Glucose was assayed in duplicate 100-

Fig. 1 Effects of food restriction on blood glucose (a) and insulin levels (b) at dusk. In the control group (circles), blood glucose falls and blood insulin rises significantly at dusk ($P < 0.01$ in each case, *t*-test with 14 d.f.). There is no significant change in either variable (squares on each graph) if food intake is restricted ($P > 0.1$ in each case, *t*-test with 14 d.f.).

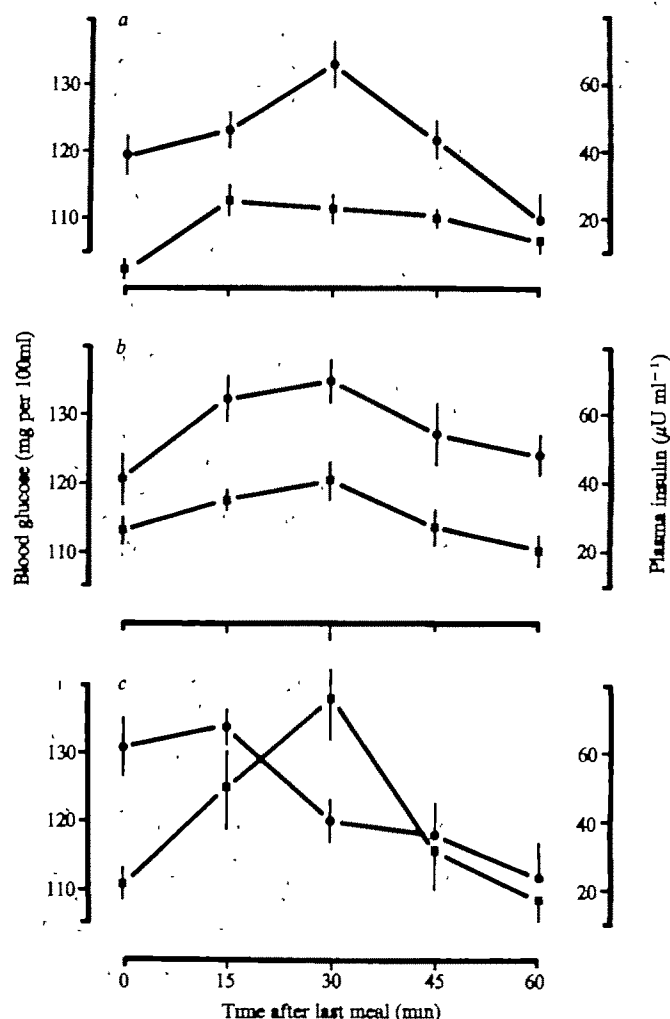
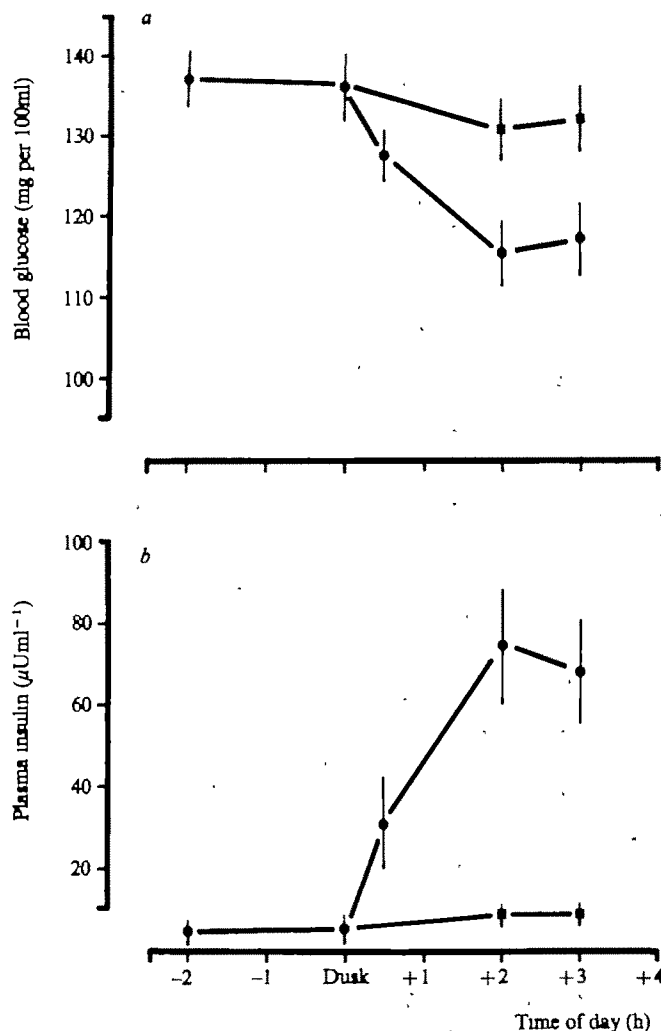


Fig. 2 Effects of combinations of meals on blood glucose and insulin levels. Each graph shows blood glucose (circles) and insulin (squares) levels at 15-min intervals after one (a), two (b) or three (c) meals taken at regular intervals around dusk. Each glucose point is the mean of 12 observations and each insulin the mean of 8. Glucose levels rise significantly only after one meal ($P < 0.01$, *t*-test 22 d.f.), and fall significantly after the third ($P < 0.01$, *t*-test 22 d.f.). Blood insulin levels rise significantly after all combinations of meals, although the magnitude of the rise clearly varies ($P < 0.01$, *t*-test with 14 d.f. in each case).

μl samples of whole blood, using a standard glucose oxidase technique (Glucostat, Worthington). Insulin was measured by radioimmunoassay, again using a standard kit (Radiochemical Amersham), on duplicate 100- μl samples of plasma. A human insulin standard was used, but as all conclusions are drawn from comparisons of insulin levels in different groups of animals, any slight systematic error thereby introduced will not affect their validity. In any one experiment each animal was bled only once, and at least 3 weeks passed between successive bleedings of the same individual, none of which were bled more than three times.

In the first study, the hourly rate of food intake of mice was measured for a few hours before and after dusk, and blood samples were removed at regular intervals for assay. Second, a group of mice were fed normally until 1 h before dusk, and thereafter restricted to their afternoon rate of feeding. Blood glucose and insulin levels sampled at regular intervals were compared with a control group allowed *ad libitum* access to food.

Mice normally eat in meals^{6,7}, and in the final experiment the normal dusk pattern of meal taking was mimicked to examine its metabolic consequence. Mice were allowed *ad libitum* food and water until 2 h before dusk, after which food was removed.

Starting 90 min later, groups of animals were given one, two or three 'meals' by allowing 10-min periods of access to food, separated by 20 min. Animals were observed to eat about the same amount in each period, and the 5% of animals who failed to eat in any or all of the periods were discarded. Blood samples were taken from groups of animals at 15-min intervals after their last meal.

There is a fivefold increase in the hourly rate of feeding at dusk. ($P < 0.001$ t -test with 31 d.f.), and this is closely associated with a fall in blood glucose ($P < 0.01$ t -test with 34 d.f.) and a rise in blood insulin ($P < 0.01$ t -test 14 d.f.). As the rate of feeding subsequently declines, these changes are reversed. If food intake is restricted (Fig. 1), blood glucose levels do not fall, and insulin levels do not rise. In the third experiment, the three 'meals' had quite different consequences. Figure 2 shows that the first meal promotes a rise in blood glucose, with little secretion of insulin, the second a further rise in glucose with a considerable rise in insulin, and the third very high insulin levels, associated with a fall in blood glucose. Insulin levels, however, fall more rapidly after the third meal than after the previous two.

Around dusk, therefore, the rate of insulin secretion in these animals increases to an extent sufficient to depress blood glucose levels in spite of a high rate of feeding. This increase could be due either to some endogenous rhythm of pancreatic activity, or to some direct or indirect effect of the sudden increase in feeding. The disappearance of the changes when food intake is restricted would favour the latter explanation. The high rate of feeding is known⁶ to be due to a very high meal frequency (three per hour), and the final experiment shows that during a series of meals very similar to that taken spontaneously by free-feeding animals, there is a transition between the day-time pattern of elevated glucose with relatively little insulin secretion to the night-time pattern of high insulin secretion, high rate of metabolic clearance of glucose, and presumably storage of energy. The voluntary pattern of feeding adopted by mice at dusk therefore seems to promote metabolic changes leading to more rapid clearance of a meal, which may, as suggested by Le Magnen, and Toates and Booth, facilitate a greater rate of food consumption by shortening the interval between subsequent meals, thus forming a type of metabolic 'positive feedback' of feeding upon itself. There are several, as yet inseparable, ways in which this might occur. Clearly, the activity of eating frequently, the operation of the gut receiving meals in rapid succession, or the metabolic effects of feeding at a high rate provide some additional stimulus to the pancreas, leading to a higher rate of insulin secretion at a given blood glucose level. The pancreatic islets are known to be influenced by both the nervous system and the gut hormones, so that either or both may contribute to the effect⁸.

If such a positive feedback could be shown to operate in other species, such as our own, then its inappropriate activation by frequent voluntary consumption at high rates may facilitate excessive weight gain.

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Cardioactive substances in the monarch butterfly and *Euploea core* reared on leaf-free artificial diet

THE monarch butterfly (*Danaus plexippus*) is one of the best known species which sequester and store cardiac glycosides¹⁻³ obtained directly from their larval food plants. On the other hand *Euploea core*, a related Danaid species feeding on the same family, Asclepiadaceae, is a poor storer of these substances⁴. Cardenolides are absent if the butterflies are reared on plants which do not contain them, and, in the past, all cardioactivity in the insects' body tissues has been attributed to dietary cardenolides. We now report that extracts of both larva and pupa reared on a leaf-free artificial diet⁵ proved cardioactive, slowing the action of an isolated rat heart (Langendorff preparation)⁶ and increasing the amplitude of contraction.

Specimens were extracted by grinding in a mortar with saline. The volume of saline varied depending on the weight of material, and, where possible, volumes were adjusted to give the same proportion of material to saline. The extraction mixture was centrifuged and 0.1-ml aliquots of this supernatant were injected into the perfusion feed line of the isolated rat heart (Langendorff) preparation. The number of hearts used and the weight of material extracted per 0.1 ml of saline are indicated in Table 1. The maximum change in amplitude of contraction is expressed as a percentage of the control value, and the heart rate was measured over 10-30 s after injection and is also expressed as a percentage of control values.

The emetic effect of mascerated specimens and extracts of *D. plexippus* (at all stages of the life cycle) when fed to pigeons is well known^{2,3,7}. The sensitivity of this test is greater than that of the isolated rat heart, as the presence of small amounts of cardiac glycosides in a butterfly extract can produce delayed emesis when administered to the pigeon, although no response may be observed when the same extract is applied to the rat heart⁸.

Extracts of larvae of *D. plexippus* reared on *Asclepias curassavica* produced an effect typical of cardiac glycosides (Table 1 and Fig. 1b), that is, a marked increase in the strength of contraction, seen most clearly in the latter half of the trace. Note that the drop in heart rate, although associated with cardenolide action, is also reminiscent of the effect of potassium ions which may have been present in the extracts, or could equally be due to

Fig. 1 a, Effect of artificial diet-reared *D. plexippus* larval extract on the rate and strength of contraction of the isolated rat heart (each vertical trace represents one heart beat). b, Effect of *A. curassavica*-fed *D. plexippus* larval extract on the isolated rat heart. c, Effect of *A. curassavica* extract on the isolated rat heart. On each figure the horizontal line indicates 10 s (this is omitted in Fig. 2b); extracts were given at the beginning of the 10-s period.

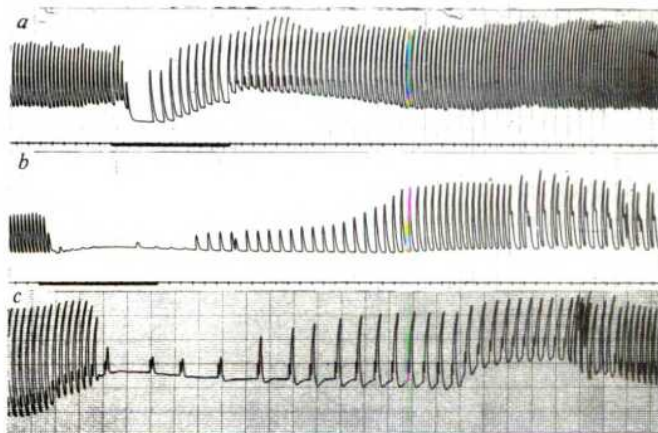


Table 1 Assay of larvae and pupae of *D. plexippus* on the rat heart

		No. of experiments	Average weight of material extracted (mg)	No. of insects extracted	% Change in Rate Amplitude	
Artificial diet fed	Larvae (2nd instar)	2	283	7	-78	+67
	Larvae (4th instar)	2	249	7	-66	+56
<i>Asclepias</i> fed	Larvae (2nd instar)	1	283	5	-97	+119
	Larvae (4th instar)	1	249	5	-98	+127
<i>Asclepias</i> fed	Pupae	2	161	2	-79	+84

Larvae were starved for 24 h before testing. Diet-reared malformed pupae did not produce emesis when fed to the pigeon whereas pupae reared on *Asclepias* did³.

the insect's own secretions. Figure 1c shows that the extract of the food plant produces a qualitatively similar effect. Comparison of this with Fig. 2c, produced by an extract from a cardenolide-free *Asclepiad* plant, *Marsdenia erecta*, shows that the latter had no discernible effect on the isolated rat heart. The same negative result was obtained with extracts from the cardenolide-free artificial diet. In contrast, extracts of larvae of both *D. plexippus* (Fig. 1a) and *E. core* (Fig. 2a) reared on the artificial diet, showed cardio-activity. The same effect was produced by extracts of the latter species reared on *Marsdenia* (Fig. 2b). However, the relatively brief slowing of the heart rate and increase in contraction are not typical of the cardenolide response elicited by *Asclepias*-reared material.

For comparison, we tested *Tyria jacobaeae*, an aposematic moth, highly repellent to vertebrate predators^{9,10}, containing large concentrations of pyrrolizidine alkaloids which it sequesters from its food plant¹¹. Neither pupa (Fig. 2d) nor foliage of the food plant on which it was reared (*Senecio jacobaeae*) produced any marked action on the rat heart.

It is unlikely that the cardioactive substance produced by *D. plexippus* is a cardenolide, despite its action on the isolated heart and the fact that some insects are known to secrete them¹², for Roeske *et al.* found no cardiac glycosides in this species reared on plants such as *Gonolobus rostratus* and *A. amplexicanlis*², which lack such substances. They did not, of course, test their

specimens for other cardio-active substances, which we can, nevertheless, assume were present. Furthermore, a substance with a very similar effect, found in *Ipomoea batatas*, and also present in the butterfly *Hypolimnas bolina* fed on it⁴, was demonstrated by Reichstein (in Dixon *et al.*)³ not to be a cardenolide.

Most highly coloured insects possess more than one chemical deterrent¹³, frequently a combination of both a primary self-secreted or self-manufactured substance, and a secondary, superimposed, plant-sequestered toxin. Such a primary defence must be particularly important in the case of *D. plexippus*, as the cardenolides which it sequesters and stores, and which so far have been considered by many workers to be its only defence mechanism, are frequently lacking in the foliage of its favourite food plants such as *A. syriaca*¹⁴. Thus, a self-secreted cardio-active substance, even though non-emetic, will make the population much less dependent on the variation and fluctuations characteristic of the secondary plant substances.

Experiments are in progress with various predators and live Danais reared on an artificial diet. It is essential to use fresh material in these feeding experiments, as, unlike the secondary plant substances, the majority of toxic self-secretions of Lepidoptera are lost or destroyed¹⁵ by storage.

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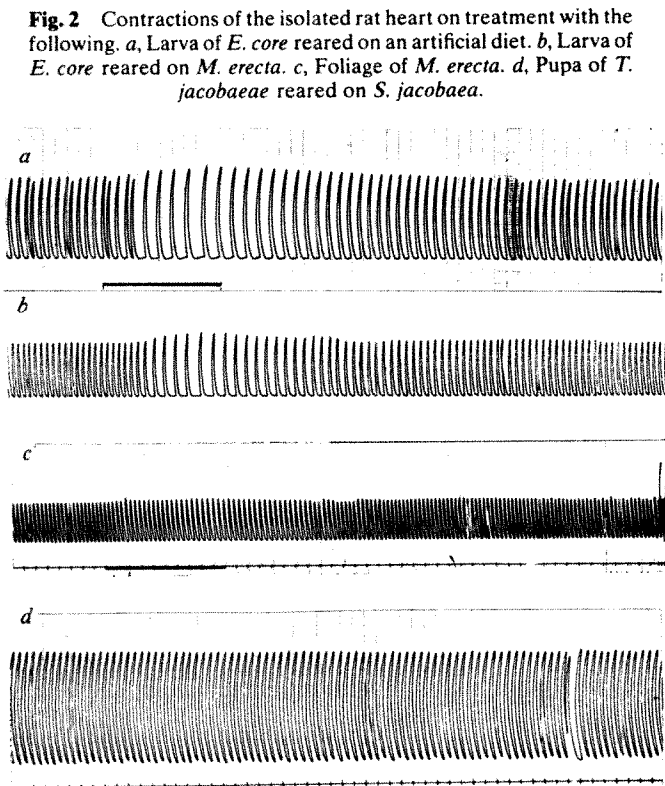
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The relationship between tonicity and flagellar length

THE manner in which a cell regulates the size and number of its organelles is not fully understood. We have studied the regulation of flagellar length. Flagella are organelles involved in cellular motility and sensory transduction¹, and in standardised conditions, the flagella of a given species are constant in length and number. Thus, taxonomists include these characteristics in species descriptions². The organism we are investigating is the unicellular biflagellate green alga *Chlamydomonas reinhardtii*, in which the two flagella are of equal length². If the flagella of *Chlamydomonas* are amputated, they regenerate to their original length in approximately 90 min (ref. 3). If only one flagellum is removed the remaining organelle shrinks while the new flagellum is elongating, until both are of the same length. At this point both organelles proceed to elongate, stopping when the normal length is reached^{3,4}. Resorption of organelles containing microtubules has been shown to occur under a number of conditions in different organisms⁵. Recently it has been shown that the flagella of *Chlamydomonas* can be caused to shorten by a variety of agents such as pyrophosphate⁶⁻⁸, chelators⁶, halothane⁹ and other salt solutions^{6,10,11}. This large variety of substances led us to study the effects of tonicity, *per se*, on flagellar length.

To determine what effect osmotic pressure has on flagellar length, vegetative cells of *Chlamydomonas reinhardtii* were incubated in two salt solutions: sodium chloride and sodium pyrophosphate, and two sugar solutions: mannitol and sucrose, all at a tonicity approximately three times that of normal growth media. Figure 1 shows that the flagella shorten to almost half their original length during the first 60 min, regardless of the solution used to alter the tonicity. Furthermore, the rate of shortening is similar in all cases. Since the only similarity between the various solutions is tonicity (not molarity), we attribute the shortening to the increased osmotic pressure.

After 60 min the flagella of cells which are in salt continue to shorten, until a steady state is reached. However, the flagella of cells which are in sugar begin to regrow, reaching near normal lengths after six h. This regrowth of flagella in the sugar can be prevented by placing the cells in the dark (data not shown). This phenomenon is under investigation.

For any given solution, the length of the flagella, at 60 min, appears to be inversely related to the tonicity of the surrounding medium. This is shown in Fig. 2a and b. Figure 2a presents data for NaCl at various tonicities and Fig. 2b presents data for sucrose at different tonicities.

The experiments in Figs 1 and 2 were of short duration (6 h), and it was of interest to determine if in continuous culture in salt solutions of different tonicities, the cells would adapt to the increased osmotic pressure and possess flagella of normal

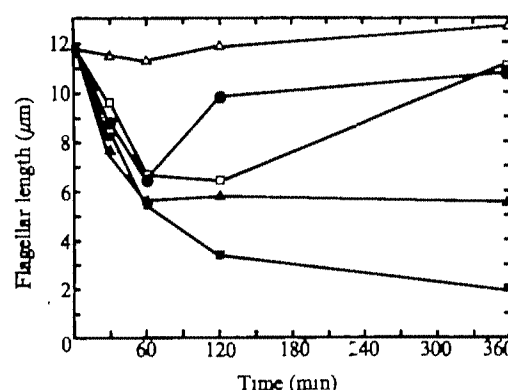


Fig. 1 Change in flagellar length at increased osmotic pressure. Vegetative cells of *Chlamydomonas reinhardtii* were grown on a 12 h light–12 h dark cycle as previously described^{1,12,13}. An equal volume of a cell suspension at a concentration of 3×10^7 cells per ml were mixed with solutions of either mannitol, sucrose, sodium chloride or sodium pyrophosphate (made up in growth media with the pH adjusted to 7.0). At timed intervals thereafter, aliquots of the cellular suspension were removed, fixed with 2.5% glutaraldehyde and flagellar lengths measured as previously described¹³. In addition, aliquots were centrifuged to remove the cells and the osmotic pressure of the supernatant was measured on a model 5100 vapour pressure osmometer (Wescor). □ Mannitol (260 mOsm kg⁻¹; 0.25 M); ● sucrose (235 mOsm kg⁻¹; 0.2 M); ▲ sodium chloride (240 mOsm kg⁻¹; 0.125 M); ■ sodium pyrophosphate (257 mOsm kg⁻¹; 0.05 M); △, normal growth medium (81 mOsm kg⁻¹).

length. The data presented in Table 1 show that the flagella of cells grown for 2 weeks in various NaCl concentrations were shorter than normal, and the higher the salt concentration, the shorter the flagella. It should be noted that at higher salt concentrations (0.1 M and higher) the rate of cell division was partially inhibited, indicating a stress condition. Thus, flagellar length might be an indicator of osmotic stress on the cells. Furthermore, the short flagella at higher salt concentrations were not a result of mutant selection, since cells washed free of salt regenerated longer flagella (data not shown).

The evidence presented here indicates that the flagella of *Chlamydomonas reinhardtii* respond to changes in osmotic pressure. Higher osmotic pressures (increased by either salt or sugar) causes shortening of the flagella. Thus we conclude that flagellar length is, in part, controlled by the osmotic pressure of the medium. This conclusion is further substantiated by the work of Telser⁹, in which flagella of *Chlamydomonas reinhardtii* were observed to elongate when the cells were placed in distilled water. We have repeated those experiments with similar results. Also, increased osmotic pressure has been shown to lead to the stoppage of the ciliary beat and ultrastructural changes in the cytosol of mussel gill cilia¹⁴. We speculate that some of the flagellar length mutants of *Chlamydomonas reinhardtii*^{15,16} might be osmoregulatory mutants; the cells may be altered in their ability to osmoregulate and therefore have shorter or longer flagella than normal.

We do not yet know the mechanism of shortening due to increased osmotic pressure. One possibility is the action of high tonicities on the microtubules forming the axoneme of the flagella. Protein turnover has been demonstrated in full length flagella, and it appears that the organelle is maintained in a state of dynamic equilibrium¹⁷⁻¹⁹. The loss of water from the cells (due to the osmotic stress) could change the concentrations of solutes in the cell and flagella, which, in turn, would alter the polymerisation and depolymerisation of microtubules.

The regulation of the length of flagella is obviously under the control of complex metabolic processes. Osmotic pressure seems to be an environmental factor which can influence those processes. However, not all flagellar shortenings are due to osmotic effects. Drugs, such as halothane⁹ and herbicides such as amiprophosmethyl⁶ probably cause flagellar shortening by

Table 1 Flagellar length of cells grown in various salt concentrations

Salt concentration (osmolarity)	Flagellar length (µm)	Per cent reduction in flagellar length
Normal medium (81)	13.3	—
0.02 M (90)	6.5	51
0.05 M (116)	4.4	67
0.1 M (196)	1.2	91

Flasks containing 50 ml of culture media, at various salt concentrations, were inoculated with 2×10^6 cells and placed to grow on a 12 h light–12 h dark cycle. After two weeks samples were removed, fixed with glutaraldehyde and flagellar lengths measured as in Fig. 1. Osmolarities were measured as in Fig. 1.

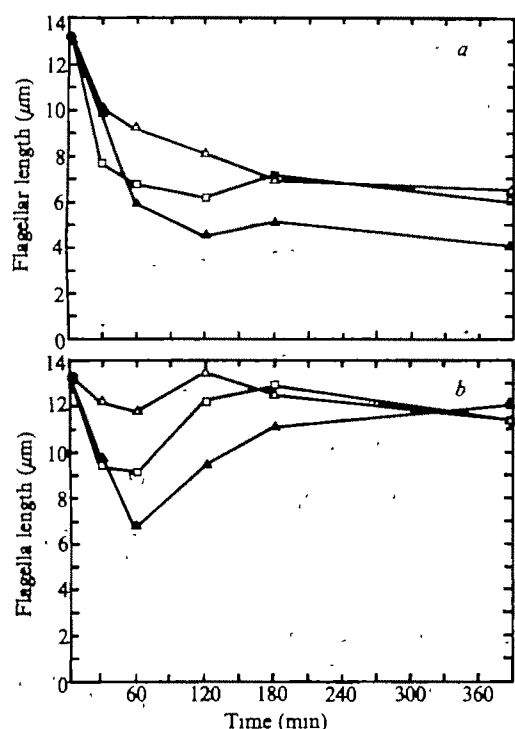


Fig. 2 Change in flagellar length in solutions of different osmolarities. Conditions of cell growth and experimental procedure were the same as in Fig. 1. *a*, Changes in flagellar length in sodium chloride solutions of various osmolarities: Δ , 163 mOsm kg⁻¹ (0.075 M); $\square$, 221 mOsm kg⁻¹ (0.1 M); $\blacktriangle$, 256 mOsm kg⁻¹ (0.125 M). *b* shows changes in flagellar length in sucrose solutions of various osmolarities: Δ , 153 mOsm kg⁻¹ (0.125 M); $\square$, 193 mOsm kg⁻¹ (0.175 M); $\blacktriangle$, 229 mOsm kg⁻¹ (0.2 M). Normal growth medium has an osmolarity of 81 mOsm kg⁻¹. The molarities of the solutions given above are in addition to normal medium.

specific chemical action. Innate cellular properties are also involved in control of flagellar length as illustrated by the alga *Euglena gracilis* which has two flagella of unequal length²⁰.

Our results are significant not only to the cell biologist interested in the physiology of the flagellum, but are also directly related to algal classification, where flagellar length is often included as a species characterisation². Attention should be paid to the salinity of the environment when comparisons are made of flagellar lengths of presumed different species.

After submission of this manuscript a paper which dealt with flagella shortening in *Chlamydomonas* was published²¹. It was reported that 150 mM NaCl caused shortening of flagella while equimolar KCl did not. Our unpublished results show that equimolar KCl will cause flagella to shorten. However, the flagella re-elongate (as in the presence of sugar) and by 90 min (the time at which flagella were measured by Quader *et al.*²¹) the flagella are of normal length. In addition we find that during re-elongation of flagella in the presence of sugar there is a simultaneous accumulation of potassium by the cells. The relationship of potassium to flagellar re-elongation is under study.

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Degenerating nerve products affect innervated muscle fibres

THE main factor controlling mammalian muscle fibre membrane characteristics is the fibre's activity^{1,2}. Other influences may be a trophic factor³⁻⁵, and a loosely termed 'inflammatory effect'^{1,6}. The latter effect is unspecific, appearing when a thread or degenerating nerve is placed over the muscle⁶, after operative procedures near the muscle (ref. 2 and our unpublished observations) and after axonal degeneration within the muscle's own nerve^{1,7,8}. This inflammatory effect may underlie the observations of Cangiano and Lutzemberger⁷ that the innervated fibres in a partially denervated muscle show certain membrane changes similar to those seen on inactive muscle fibres. Such surface changes in inactive muscles have been implicated as the cause of α motoneuronal terminal sprouting⁹, and that they may also occur on innervated fibres in partially denervated muscles provides an explanation for the occurrence of terminal sprouting here too without invoking additional mechanisms. However Cangiano and Lutzemberger's results have been disputed¹⁰. We now report an experiment which demonstrates that degenerating nerve fibres can affect innervated muscle fibres, and that this interaction may well stimulate motoneuronal terminal sprouting.

We produced muscles with afferent nerve fibre degeneration but with normal efferent innervation by cutting the dorsal roots supplying the muscles, peripheral to the dorsal root ganglia. We used the soleus and peroneus tertius muscles of mice weighing 30 g or more. Under chloral hydrate anaesthesia (0.2 ml of 3.5% solution per 10 g body weight) the L4 and L5 spinal foraminae were exposed and as much of the dorsal root ganglia as possible removed, care being taken to avoid damaging the ventral roots. Five to eight days later (when any inflammatory effect should be present¹) the acute experiment was performed, the muscles being examined *in vitro* using methods described previously¹¹. We confirmed the absence of motor denervation by comparing the nerve and directly evoked twitch and 50 Hz, tetanic tensions. We also counted the number of motor units in the muscle using carefully graded stimuli to the nerve and compared this total with that obtained from contralateral control muscles. Muscles with any indication of motoneurone death were discarded (8 muscles from 38 operations). A muscle's acetylcholine sensitivity was taken as the size of its contracture in 5×10^{-4} g ml⁻¹ acetylcholine perchlorate, expressed as a percentage of its maximum directly evoked 50 Hz tetanus. The muscles with their nerves were stained in zinc iodide and osmium tetroxide, and the amounts of degeneration within the nerve, de-afferented muscle spindles, and motoneuronal sprouting were determined.

Controls consisted of the normal contralateral muscles from experimental animals, and also muscles from mice with dorsal roots cut central to the ganglion and hence with intact afferent axons (rhizotomy). This latter group controls for the effects of removing some afferent input to the spinal cord on muscle activity, and also for any effects of nerve degeneration within the spinal cord.

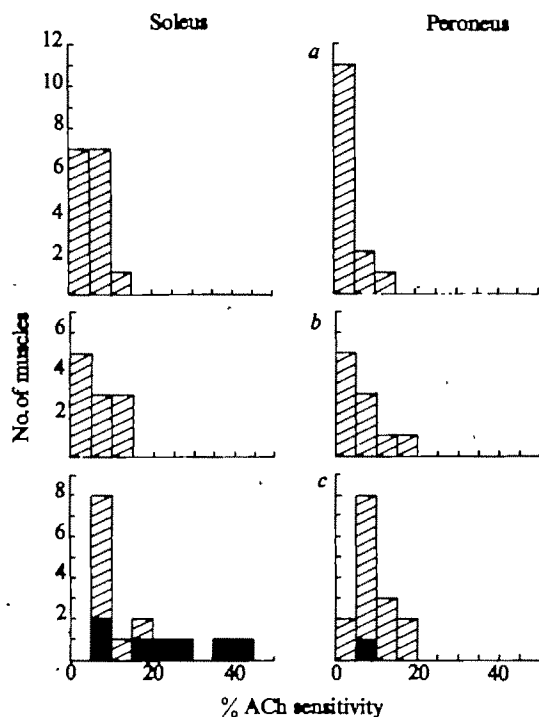


Fig. 1 Histograms of the acetylcholine sensitivities of the various groups of muscles. The rhizotomised solei (*b*) are not significantly different from the normal control muscles (*a*). However, the ganglionectomised solei (*c*) are significantly different from normal muscles ($P < 0.001$ Mann-Whitney *U* test), and significantly different from the rhizotomised control muscles ($P < 0.025$). The rhizotomised peronei are significantly different from normal controls ($P < 0.05$); as are the ganglionectomised peronei ($P < 0.01$). The two operative procedures do not produce significantly different acetylcholine sensitivities in this muscle, although the mean sensitivity is higher in the ganglionectomies (10 ± 0.5 , $n = 15$ compared with 7 ± 1.6 , $n = 10$). The black portions on the histograms are the results from muscles where more than 40% of the spindles were de-afferented.

Figure 1 shows the acetylcholine sensitivity of the three groups of muscles. The ganglionectomised solei are significantly more sensitive than either of the control groups ($P < 0.025$, Mann-Whitney *U* test), which are not significantly different from one another. Both the ganglionectomised and rhizotomised peronei are significantly more sensitive than the control normal muscles ($P < 0.01$ and $P < 0.05$, Mann-Whitney *U* tests). In this muscle the two operations do not produce significantly different effects, though ganglionectomy does produce the higher mean sensitivity (10 ± 0.5 , $n = 15$ compared with 7 ± 1.6 , $n = 10$, mean $\pm$ s.e.m.). Figure 2*a* shows a scattergram of the relationship between the acetylcholine sensitivity and the amount of de-afferentation within soleus. The correlation ($r_s = 0.525$) is significant ($P < 0.05$). Thus, particularly in the soleus, nerve degeneration products do seem to cause innervated muscle fibres to become acetylcholine sensitive.

Examination of the α -motoneurone terminals in experimental muscles reveals terminal sprouting. Figure 2*b* is a scattergram of the relationship between terminal sprouting and de-afferentation in soleus, and there is a significant correlation ($r_s = 0.453$, $P < 0.05$). The percentage of terminals with sprouts was 17 ± 5.6 , $n = 16$ in ganglionectomised solei, 6 ± 1.0 , $n = 9$ in rhizotomised solei and 7 ± 2.8 , $n = 10$ in normal solei.

These results support the suggestion that muscle fibre surface changes are a local stimulus to terminal sprouting⁹, and agree with the results of other experiments¹². However, we cannot rule out the possibility that some feature of degenerating nerves might act directly on intact nerve fibres to stimulate terminal sprouting, as seems to be the case for collateral or nodal sprouting¹³. We do in fact see some collateral sprouting in the

intramuscular nerve branches of these de-afferented preparations.

Extensive de-afferentation is required to give significant increases in acetylcholine sensitivity (Fig. 2*a*) and it is only in such muscles that we see much sprouting (Fig. 2*b*). However, it is well known that relatively small amounts of partial motor denervation are sufficient to produce large increases in sprouting. In fact, removing only one or two motor units from soleus by selective ventral root damage can produce up to 60% terminal sprouting (data not shown). This might suggest that muscle fibres or motoneurons are especially responsive to motoneurone degeneration. A simpler explanation is that motoneurons arborise profusely within a muscle and pass close to one another's endplates, unlike afferent fibres which do not branch much nor run through the endplate zone. Consequently a single degenerating efferent fibre may produce larger amounts of active substances closer to their sites of action than a single degenerating afferent. A third possible explanation for the apparently greater amount of sprouting after ventral root damage is that denervated muscle fibres might liberate a sprouting agent which could spread through the muscle. This seems unlikely, because in a muscle where transmission is partially blocked, sprouting occurs only from the blocked terminals⁹. If the inactive and hence altered muscle fibres liberated diffusible chemicals which could elicit sprouting then one might expect unblocked terminals to sprout also.

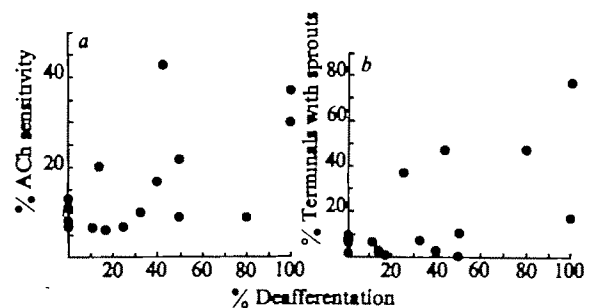


Fig. 2 *a*, A scattergram of the relationship between acetylcholine sensitivity of solei and the amount of de-afferentation produced in ganglionectomised preparations, r_s (Spearman rank correlation coefficient) = 0.525, which is significant ($P < 0.05$). The degree of de-afferentation was assessed by counting the number of deafferented muscle spindles and expressing this as a percentage of the total number of spindles in that muscle. A normal soleus has 6–8 spindles. Obviously this may only be a rough estimate of the total amount of de-afferentation. This graph demonstrates that large amounts of de-afferentation must be produced to have much effect on the muscle's acetylcholine sensitivity, and this may explain the small size of the differences seen in Fig. 1. Especially in the earlier experiments, in trying to avoid ventral root damage the surgery was over cautious and removed rather little of the ganglia. Histologically, only small amounts of degeneration were seen, and the proportion of de-afferented spindles was small. This problem was particularly acute in the peroneus, because this muscle appears to receive the bulk of its afferents through L5, and this ganglion being harder to remove is usually less damaged. The result was that fewer than half the peronei had any completely de-afferented spindles. Considering only the black squares in Fig. 1 (de-afferentation > 40%), the effect of ganglionectomy is quite obvious. (The rhizotomy operation is relatively easy, and always destroys 100% of the limb's afferent input to the spinal cord.) The particularly small effect of ganglionectomy in peroneus might also be because it contains 'fast' motor units. It has been shown that a fast pattern of muscle activity is particularly effective in inhibiting the development of acetylcholine supersensitivity¹. *b*, A scattergram of the relationship between terminal sprouting and de-afferentation. There is a significant correlation ($r_s = 0.453$, $P < 0.05$). There is also a significant correlation between the amount of sprouting and the acetylcholine sensitivity of the muscle ($r_s = 0.523$, $P < 0.05$), as is found in certain other circumstances in which sprouting occurs^{9,12}.

Finally, in order to unravel complex innervation patterns (for example, those of the muscle spindle), it is common to use de-afferented or de-efferented preparations. Our experiments have shown that de-afferentation causes efferent sprouting; clearly this may produce misleading descriptions of the normal pattern of innervation in such preparations. Perhaps this explains some of the controversy¹⁴ over the innervation of the muscle spindle.

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Adjuvant-induced thymus-derived suppressor cells of cell-mediated tumour immunity

THE use of immunoadjuvants in enhancing immune responses to various antigens and particularly in tumour therapy is based largely on the concept that an increased level of specific immunity may be achieved by nonspecific stimulation of the immune system¹. However, the tumour-protective effect of adjuvants has not been entirely consistent in all systems studied, and also, an immune suppressive effect of adjuvants has been demonstrated mainly by using *in vitro* assays of cell-mediated immunity². It remains to be determined whether a defect measured by such general tests of lymphocyte function may also represent an indication of depressed cell-mediated immunity against the progressive growth of tumours. Moreover, the target cell in the immune system for adjuvant effect has not been characterised clearly. Our laboratory has made extensive study of immune response to SV40-induced syngeneic transplantable MKSA cells of BALB/c mice³⁻⁵. This tumour has been shown to possess tumour-associated antigens which can immunise specifically against the growth of MKSA cells. The role of T lymphocytes in the immune response against SV40-induced tumour-associated antigens was shown by *in vivo* tumour cell neutralisation assay (Winn)⁶ and by *in vitro* ⁵¹Cr release assay⁷. We now report that injection of complete Freund's adjuvant (CFA), one of the most useful adjuvants in enhancing immune responses, markedly inhibited the ability of immune spleen cells to prevent SV40-induced tumour growth in the Winn assay and to lyse SV40-transformed cells in the cytotoxicity assay. Moreover, untreated mice which reject suboptimal numbers of tumour cells die of progressive tumour growth following treatment with CFA and tumour cells; adult thymectomy reverses the suppression caused by CFA; and the target cell for CFA effect is the T lymphocyte. These data suggest that treatment

Table 1 The effect of CFA on growth of SV40-induced tumour and on cell-mediated cytotoxicity against SV40-transformed cells

Treatment	Challenge with MKSA cells* (dead/total)	Neutralisation of MKSA cells† (dead/total)	% Cytotoxicity against C57SV cells‡
None	0/10	0/10	39.2
CFA	9/10	10/10	2.3

This is a representative experiment out of five experiments with similar results.

* CBF₁ mice were inoculated s.c. with 2 × 10⁷ C57SV cells. After 15 d the animals were challenged s.c. with 1 × 10⁵ MKSA cells.

† CBF₁ mice were inoculated s.c. with 2 × 10⁷ C57SV cells. After 15 d the spleens were removed and a T-cell-enriched fraction was prepared as described earlier. The cells were incubated with 1 × 10⁵ MKSA cells, at a 300:1 lymphocyte:tumour cell ratio, for 45 min at 37 °C in a CO₂ incubator and injected s.c. into normal CBF₁ mice.

‡ CBF₁ mice were inoculated s.c. with 2 × 10⁷ C57SV cells. After 8 d a T-cell-enriched fraction was prepared from the spleens of these mice and used in the *in vitro* ⁵¹Cr release assay. The assay was carried out in the wells of flat-bottom microplates. C57SV target cells were trypsinised and 1 × 10⁴ cells and 5 µCi Na⁵¹CrO₄ in 0.2 ml RPMI 1640 medium containing 5% fetal bovine serum were added to each well. After 18 h at 37 °C in a CO₂ incubator, the target cells were washed and effector cells were added in a 0.2 ml volume in a ratio of 200:1 lymphocyte:target cell. After 6 h incubation at 37 °C in a CO₂ incubator, the plates were centrifuged at 350g for 5 min and 0.1 ml of the supernatant was removed for counting. results are calculated from the mean of triplicate samples and expressed as:

% Specific lysis

$$= \left(\frac{\text{c.p.m. } ^{51}\text{Cr released in the presence of immune cells}}{\text{c.p.m. } ^{51}\text{Cr released in the presence of normal cells} - \text{c.p.m. } ^{51}\text{Cr released in the presence of 1\% SDS}} \right) \times 100$$

The ⁵¹Cr released in the presence of normal cells was always either very close or identical to the release in medium and ranged over 5-15%.

with CFA causes depression of specific anti-tumour cell-mediated immunity by activation of suppressor thymus-derived lymphocytes.

Female CBF₁ (BALB/c × C57BL/6)F₁ mice aged 8-12 weeks (unless otherwise indicated) were used and SV40-transformed cells, C57SV, of C57BL/6 mice. These cells are good targets in the *in vitro* ⁵¹Cr release assay^{7,8} but do not grow *in vivo*. SV40-transformed cells, MKSA, of BALB/c mice grow *in vivo*⁹ but are poor targets *in vitro*. Both cell lines were shown to possess tumour-associated transplantation antigens which can immunise specifically against the growth of MKSA cells^{4,8}.

In the first experiment the mice were inoculated intraperitoneally (i.p.) with 0.5 ml of CFA (0.5 mg ml⁻¹ *Mycobacterium tuberculosis*, Difco) or left untreated, and immediately afterwards they were inoculated subcutaneously (s.c.) with 2 × 10⁷ C57SV cells. (The amount of CFA and the timing of inoculation in relation to C57SV cell immunisation were based on preliminary experiments.) The mice were then divided into three groups. The spleens of the first group of mice were removed 8 d after immunisation^{7,8}, and a T-cell-enriched fraction was prepared using nylon wool columns, as described by Julius *et al.*¹⁰. About 30% of the starting spleen cell population was recovered in the nonadherent fraction; of these, 1-3% (compared to 40-45% in the unfractionated population) were surface immunoglobulin-positive cells, and 0.3% were phagocytic cells (compared to about 5% in the unfractionated population) as judged by latex particle ingestion. The cells were used in the ⁵¹Cr release assay against C57SV target cells. Marked depression of cell-mediated cytotoxic activity was observed in the CFA-treated group as compared to the control group (Table 1). This suppression was demonstrated by using various attacker:target cell ratios and by testing spleen cells at various times after C57SV cell immunisation. The second group of mice was challenged s.c. with 1 × 10⁵ MKSA cells 15 d after immunisation.

Table 2 The effect of CFA-induced suppressor T cells on growth of SV40-induced tumours

Treatment and no. of spleen cells transferred		Challenge with MKSA cells† (dead/total)
Untreated unfractionated	6×10^7	0/10
CFA-treated unfractionated	6×10^7	8/10
CFA-treated unfractionated	3×10^7	1/10
CFA-treated unfractionated	1.5×10^7	1/10
Untreated nylon column	6×10^7	1/10
CFA-treated nylon column	6×10^7	9/10
CFA-treated nylon column	3×10^7	8/10
CFA-treated nylon column	1.5×10^7	1/10
Untreated anti- θ and complement (C)*	6×10^7	2/10
CFA treated anti- θ and C	6×10^7	0/10
CFA treated anti- θ and C	3×10^7	1/10
CFA treated anti- θ and C	1.5×10^7	1/10
CFA treated C alone	6×10^7	8/10

* The preparation of AKR anti-Thy 1.2 C3H antibody and the treatment of lymphoid cells were carried out according to the technique described in detail in ref. 12. This antiserum lysed more than 97% of C3H thymocytes and 35–40% of C3H spleen cells by trypan blue dye exclusion. The T-cell response of spleen cells to phytohaemagglutinin was abolished after treatment, whereas the B-cell response to lipopolysaccharide was left intact.

† CBF₁ mice were inoculated i.v. with various spleen cell preparation as shown and immediately s.c. with 1×10^5 MKSA cells.

The third group was killed 15 d after immunisation and T-enriched spleen cell populations were mixed with 1×10^5 MKSA cells and injected s.c. into normal CBF₁ mice. All the mice were inspected until death from tumour growth.

In both groups, CFA treatment caused suppression of tumour immunity (Table 1); thus, most CFA-treated mice died of progressive tumour growth after challenge whereas no tumours were observed in untreated animals and no animal died. Similarly, in the Winn assay, inhibition of tumour cell neutralisation activity of lymphocytes from CFA-treated mice resulted in tumour growth and death of all animals as opposed to untreated animals, in which tumour cell neutralisation resulted in complete protection against tumour growth. Similar results were observed at various times after immunisation and by using various lymphocyte:tumour cell ratios. Incomplete Freund's adjuvant did not have such an effect. No animal died if inoculated with CFA alone. These experiments suggested that suppression of specific cell-mediated tumour immunity may be caused by either a defect in T-cell function or by activation of suppressor cells. To test these hypotheses further, the following *in vivo* experiment was carried out. Normal CBF₁ mice were inoculated i.p. with 0.5 ml of CFA or left untreated. Seven days later (a time based on preliminary experiments), the animals were killed and their spleens were either left unfractionated, passed through nylon wool columns to remove B cells and phagocytic cells, or treated with specific anti-Thy 1.2 serum and complement to remove T cells. The various spleen cell populations were inoculated intravenously (i.v.) into normal CBF₁ mice which were then injected s.c. with 1×10^5 MKSA cells (the number of tumour cells which in preliminary experiments was found to result in no tumour growth in normal CBF₁ mice as opposed to a higher number of cells). The results of a representative experiment out of two experiments with similar results are summarised in Table 2. Injection of mice with spleen cells from untreated animals, either unfractionated or enriched for T cells or B cells and macrophages, and challenge with MKSA cells did not result in tumour growth or death of these animals. On the other hand, injection of mice with spleen cells from mice pretreated with CFA, either unfractionated or enriched for T cells, resulted in tumour growth and death of the animals.

Treatment of cells with specific anti-Thy 1.2 serum and complement but not with complement alone abolished the suppressive activity. These results suggested that suppressor T cells were generated in CFA-treated animals and suppressed *in vivo* the immune response against MKSA cells.

A similar experiment was carried out using adult thymectomy, as it was shown that adult thymus may actively regulate the function of T cells¹¹. Normal CBF₁ mice 6–8 weeks old were either sham thymectomised, thymectomised or left untreated. Ten days later the mice were inoculated i.p. with 0.5 ml CFA or were left untreated, and all mice were inoculated s.c. with 1×10^5 MKSA cells. The results of a representative experiment out of three experiments with similar results are summarised in Table 3. Normal mice treated with CFA died of tumour growth as compared to nontreated animals. Similar results were obtained with sham thymectomised mice. On the other hand, thymectomy and treatment with CFA did not result in tumour growth, similar to results with thymectomy without CFA treatment. Thus, adult thymectomy reversed the suppressive effect of CFA, and protected mice from the lethal effect of tumour growth.

Table 3 The effect of adult thymectomy and CFA on growth of SV40-induced tumour

Treatment	CFA	Challenge with MKSA cells* (dead/total)
None	—	1/10
None	+	9/10
Sham thymectomy	—	0/10
Sham thymectomy	+	8/10
Thymectomy	—	2/10
Thymectomy	+	1/10

* CBF₁ mice were treated as shown and described in the text and inoculated s.c. with 1×10^5 MKSA cells.

The present study demonstrates the suppression of anti-tumour cellular immunity by treatment with CFA, as assessed by *in vivo* tumour rejection and *in vitro* cell-mediated cytotoxicity, two immunological functions mediated by T cells. CBF₁ mice challenged with syngeneic SV40-transformed cells and CFA failed to develop effector cells, and instead they developed suppressor T cells which inhibited *in vivo* effector T-cell functions. In contrast, adult thymectomy reversed the suppression caused by CFA, suggesting that the intact adult thymus was necessary for the induction of suppressor cells, that is, the thymus may regulate immune responsiveness by the export of suppressor T cells which regulate other T-cell responses. These studies agree with those demonstrating suppression of *in vitro* immune responses to alloantigens by CFA^{13–15}. In contrast to suppressing effector-cell function to antigens, CFA augmented antibody responses to the same antigens^{16,17}. Those studies suggested that increased helper T-cell activity is favoured by adjuvant. Taken together, these and our own studies suggest that CFA has a differential effect on at least two T-cell subpopulations. Regulation of T-cell function is accomplished by the induction of suppressor T cells that selectively interact with pre-killer T cells while sparing helper T cells. A relevant aspect of tumour immunology suggested by the present study is that there may be quite a delicate balance between the beneficial anti-tumour effects of adjuvants and their immunosuppressive effects. Considerable experimentation seems to be necessary to determine the various factors contributing to this complex situation; therefore, caution should be exercised in using adjuvants for immunotherapy of cancer.

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Characterisation of a tumour-specific antigen on the surface of feline lymphosarcoma cells

IN domestic cats, regression of tumours originally induced by inoculation of feline sarcoma virus (FeSV) as well as the resistance to feline leukaemia virus (FeLV)-induced lymphosarcoma in the natural environment are correlated with high titres of antibody to the feline oncornavirus-associated cell membrane antigen (FOCMA)¹⁻⁴. FOCMA is present on FeLV-producing and nonproducing feline lymphosarcoma (LSA) cells and FeSV-infected fibrosarcoma cells but is absent from FeLV-infected normal cells. This finding is consistent with the interpretation that FOCMA represents an FeLV or FeSV-induced transformation-specific protein⁵⁻⁷. Other workers have initiated studies of the biochemical nature of FOCMA derived from cytoplasmic extracts of cells of a heterologous species (mink) nonproductively transformed by FeSV⁸⁻¹⁰. However, our primary interest has been to analyse FOCMA expressed on naturally occurring LSA cells from pet cats. We describe here the identification of a hitherto unrecognised protein on the surface of feline LSA cells and cultured transformed feline lymphoblastoid cells which exhibits antigenic determinants in common with FOCMA. The molecular weight of this protein is approximately 70,000. Our data agree with the accumulating evidence that FOCMA is distinct from all FeLV and RD114-coded virion antigens^{4,6-11}.

Cell-surface molecules carrying FOCMA determinants were characterised by methods previously used in studies of murine leukaemia virus-coded cell-surface components of normal and leukaemic murine lymphoid cells: enzymatic radioiodination of viable cells, precipitation with typing serum from detergent lysates, and analysis of proteins in immunoprecipitates by polyacrylamide gel electrophoresis in the presence of sodium dodecyl sulphate (SDS-PAGE)^{12,13}. Correct interpretation of the results obtained was dependent on the availability of monospecific FOCMA typing sera. Therefore, a preliminary comparative analysis of feline sera for antibodies to FOCMA and to FeLV structural protein antigens was made. A panel of selected coded sera were tested for the presence of (1) FOCMA-reactive antibodies by the viable cell membrane immunofluorescence test, (2) FeLV-neutralising antibodies, and

(3) precipitating antibodies to individually purified FeLV-AB virion proteins in radioimmunoassays. We were able to select cat sera (Table 1) which reacted only against FOCMA and not against FeLV structural protein antigens (hereafter referred to as FOCMA typing serum (from a class 6 cat)), sera reacting only against FeLV proteins (class 3 cat), or sera negative for FOCMA or FeLV antibodies (class 5 cat).

Figure 1 shows electropherograms of proteins precipitated from lysates of surface-iodinated primary feline LSA cells by sera from different classes of FeLV-exposed cats. Figure 1a and

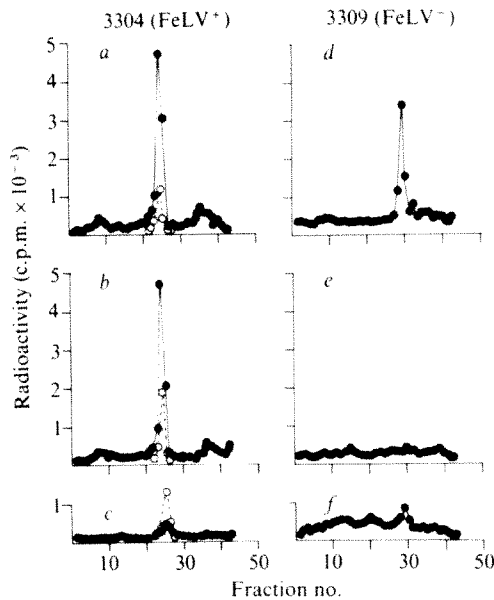
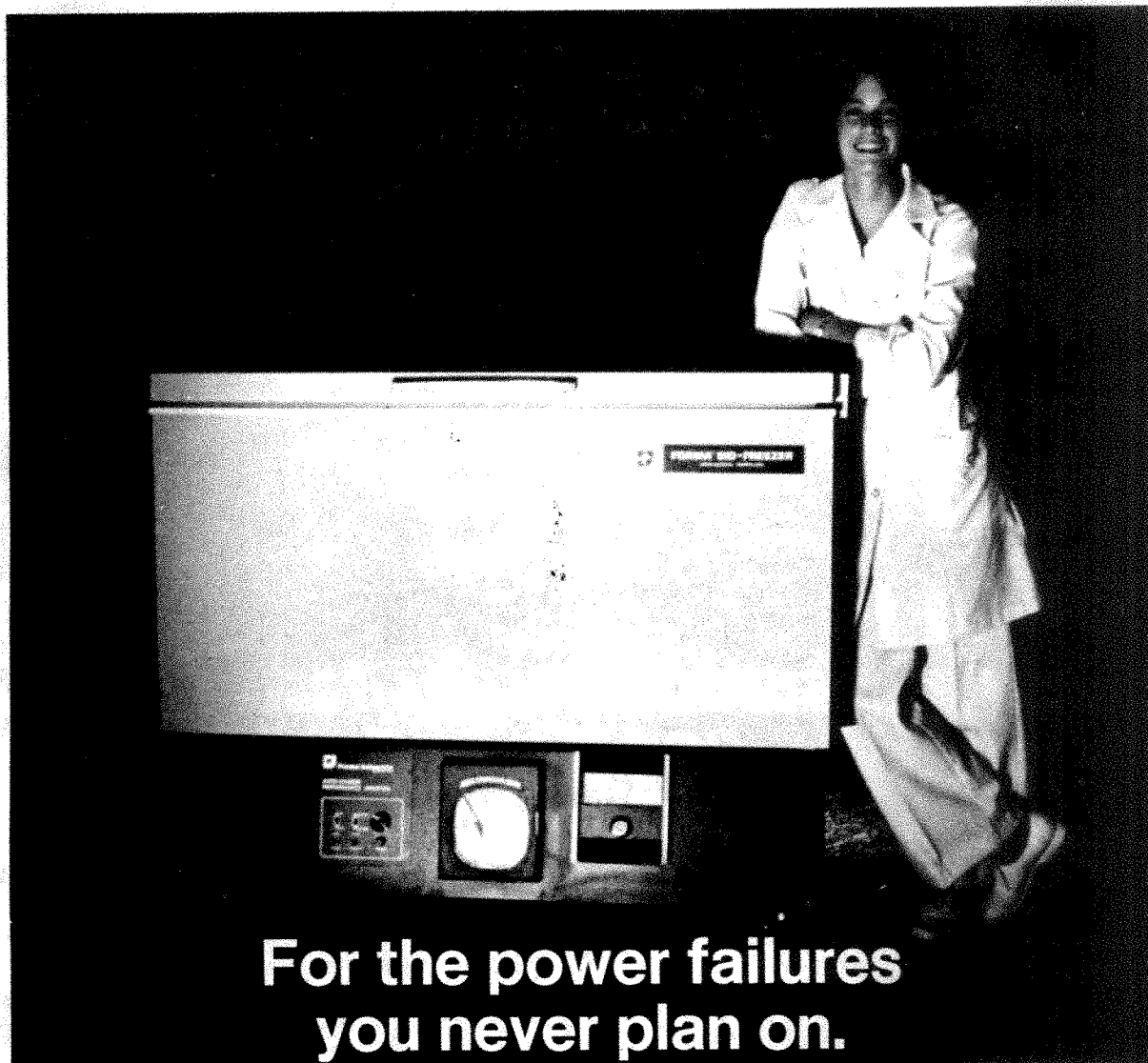


Fig. 1 SDS-polyacrylamide gel (PAG) analysis of ¹²⁵I-labelled surface proteins of primary feline lymphosarcoma (LSA) cells precipitated from cell lysates by antibodies in different classes of feline sera (●). Tumour tissue was obtained from animals at autopsy; thymic LSA cells, FeLV-positive in a fluorescent antibody test for FeLV gs antigens¹⁷, were obtained from cat no. 3304. LSA cells negative for FeLV antigen expression were obtained from a lymph node of a second animal (3309) with multicentric LSA. Preparation of cells and radioiodination of cell-surface proteins were accomplished according to protocols published in detail elsewhere¹². Labelled proteins were solubilised by incubating cells in a cell lysis buffer (CLB) (0.05 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.5% Nonidet P40, 0.5% sodium deoxycholate) containing 10% Trasylol, a general protease inhibitor (FBA Pharmaceuticals). After dialysis for 16 h at 4 °C, insoluble material was removed by centrifugation. For our double-antibody precipitation procedure, 2 µl of test feline sera was added to aliquots of lysate from 5 × 10⁶ cells (final volume 100 µl in CLB, 2-3 × 10⁶ trichloroacetic acid (TCA)-precipitable ¹²⁵I c.p.m.). The suspensions were incubated at 4 °C for 30 min and the total IgG was precipitated by further incubation (4 °C, 2 h) with an excess of rabbit anti-cat IgG. Precipitates were collected by centrifugation at 3,000g and sequentially washed with 1-ml aliquots of cold (1) 0.5 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.5% NP40, (2) 1.0 M NaCl, 0.02 M Tris-HCl, pH 7.4, 0.5% NP40, and (3) 0.002 M Tris-HCl, pH 7.4, 0.5% NP40. Labelled polypeptides in immunoprecipitates were analysed by PAG electrophoresis in the presence of SDS and 2-mercaptoethanol in 0.5 × 8.5 cm cylindrical gels (7.5% acrylamide) topped with 0.6 × 1.5 cm stacking gels (4.5% acrylamide) according to the method of Laemmli¹⁸. The ratio of acrylamide to bisacrylamide was 30:0.8. After electrophoresis at 3 mA per gel for 3 h, gels were fractionated and ¹²⁵I in the fractions was determined in a Beckman radioimmuno analyser γ counter. a-c show proteins immunoprecipitated from 3304 LSA cell lysate with FOCMA typing serum 3192A (a), FeLV-neutralising serum 2934C (b) and control cat serum 2263A (c). Proteins immunoprecipitated from 3309 LSA cell lysate with feline sera 3192A, 2934C and 2263A are shown in d, e and f, respectively. ³H-glucosamine-labelled AKR murine leukaemia virus was subjected to co-electrophoresis with immunoprecipitates to serve as a marker for viral gp70 (○).

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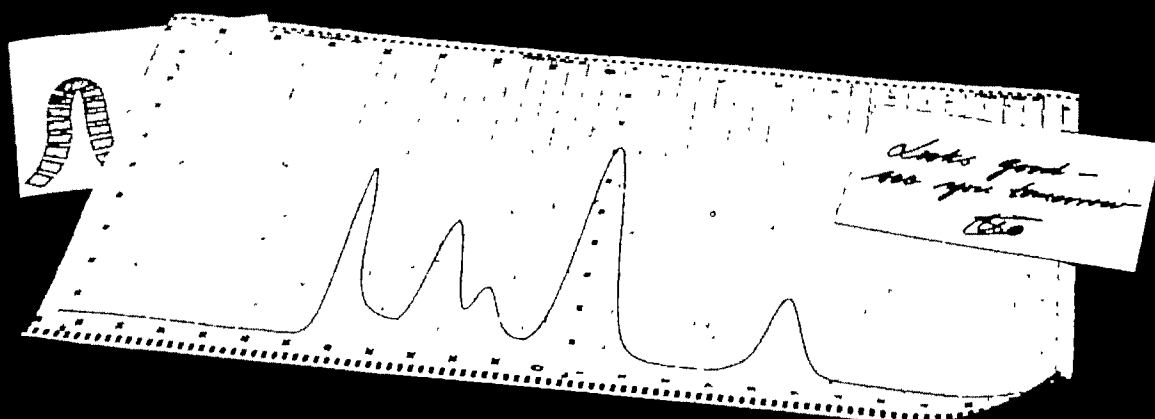


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Table 1 Antibodies in different classes of feline sera

Serum code	Class*	FeLV status†	FeLV neutralising antibody‡	FOCMA antibody§	gp70	RIA titres against ¹²⁵ I-labelled FeLV proteins	
						p30	p15
2934C	3	—	>10	<16	80	320	640
2530A	3	—	>10	<16		1,280	640
2584D	3	—	>10	<16		20	
2768B	3	—	>10	<16			80
2904C	3	—	>10	<16		40	2,560
2263A	5	+	<2	<16	<20	<20	<20
2313A	6	+	<2	32–64	<20	<20	<20
3192A	6	+	<2	128	<20	<20	<20

* Classification of Hardy *et al.*⁴. Class 1 cats are those uninfected cats not exposed to FeLV who do not have neutralising or FOCMA antibody. Classes 2, 3 and 4 cats are uninfected cats exposed to FeLV but not persistently infected with the virus; class 2 cats develop antibodies to FOCMA but no FeLV-neutralising antibodies, class 3 cats develop only neutralising antibodies and class 4 cats develop both types of antibodies. Classes 5 and 6 cats are persistently infected with FeLV; class 5 cats develop no FeLV-neutralising or FOCMA-reactive antibodies, and class 6 cats develop only antibodies to FOCMA.

† Immunofluorescent antibody tests of peripheral blood leukocytes¹⁷.

‡ Titres are the reciprocal of serum dilution giving complete neutralisation of the ability of 40 infectious units of FeLV to induce fluorescent foci in cultured feline fibroblasts.

§ Titres are reciprocals of serum dilutions staining at least 50% of 7.5×10^5 target feline transformed lymphoblastoid cells (FL74)⁶.

|| Titres are reciprocals of dilutions of monospecific rabbit immune sera still precipitating 2.5 ng of corresponding radioiodinated FeLV protein.

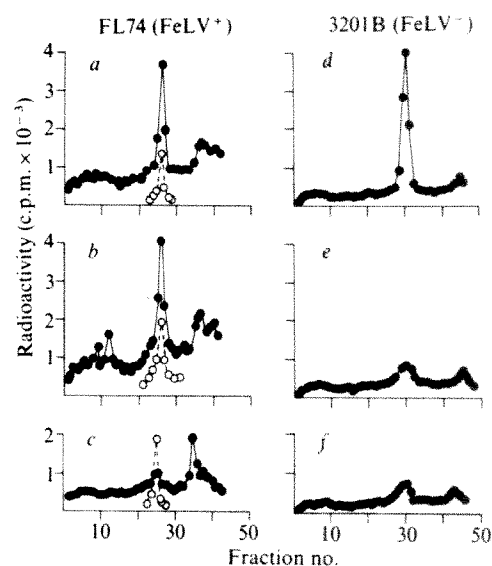
d shows that antibodies in our FOCMA typing serum precipitated a single protein species from LSA cells, regardless of whether these cells showed concomitant expression of FeLV structural antigens. In contrast, immunoprecipitation of a presumed FeLV gp70 molecule with the FeLV-neutralising serum from a class 3 cat was restricted to the FeLV-positive cells (Fig. 1*b, e*). The latter molecule was not precipitated by serum from the antibody-negative class 5 cat (Fig. 1*c, f*). The data shown are representative of results obtained in a series of experiments with biopsied tumour tissue from thymic, multicentric and alimentary forms of feline LSA. Cells from LSAs of the thymus, lymph nodes, kidneys or blood, including tumours of both T- and B-cell origin, were analysed. The molecular weights of the molecules reactive with the gp70 and FOCMA typing sera were estimated to be about 70,000 daltons by virtue of their co-migration with ³H-glucosamine-labelled AKR virus gp70 subjected to co-electrophoresis. A comparison by co-electrophoresis in slab gels of these molecules and several marker proteins of known molecular weight led to a similar conclusion.

These results suggested that the virus-neutralising serum reacted with cell-surface FeLV gp70 and the FOCMA typing serum with a similarly sized molecule possessing different antigenic characteristics. To clarify the reactivities in these sera for FeLV-producing transformed cells a standard target cell line was needed. For this, we chose the well characterised transformed feline lymphoblastoid line FL74 (ref. 5), the standard line used for detection of FOCMA antibodies in cat sera. These cells had been shown to produce FeLV of A, B and C serotypes and to express FeLV envelope and core protein antigens on their surface^{5,14}. A second transformed feline lymphoblastoid cell line (FOCMA-positive but not expressing FeLV antigens) recently established in our laboratories was also studied as a control (W.D.H. Jr *et al.*, in preparation).

In initial experiments (Fig. 2), using a radioimmuno-precipitation assay as described above, we verified that our FOCMA-reactive and FeLV-neutralising sera recognised cell surface molecules on FL74 cells which were similar to those detected on freshly biopsied tumour tissue. That the FOCMA-specific and FeLV-neutralising cat sera were in fact recognising different 70,000-dalton molecules on these cells was established in absorption experiments. Absorption of FOCMA typing serum with normal cat lymph node cells (cells from six animals were tested, including one replicating FeLV), cultured uninfected or FeLV-producing feline lung fibroblasts (FLF), or cultured heterologous mink lung fibroblasts (CCL64)¹⁵, had a negligible effect on precipitation of FOCMA. However, absorption with LSA cells (three samples of FeLV producers

and four FeLV nonproducers) or heterologous mink lung fibroblasts nonproductively transformed by FeSV (64F3CL7 cells)¹⁵ led to a sharp decrease in precipitating activity, such that electropherograms resembled that obtained with antibody-negative cat serum (for example, Fig. 2*c*). Conversely, absorption of precipitating activity in the FeLV-neutralising serum was achieved with FeLV-infected normal feline lymph node cells, cultured FLF cells and LSAs, but not with FeLV-uninfected cells of those types. In other experiments, complete absorption

Fig. 2 SDS-PAGE analysis of ¹²⁵I-labelled surface proteins of cultured transformed feline lymphoblastoid cells precipitated from cell lysates by antibodies in different classes of feline sera. The transformed feline lymphoblastoid cell lines studied were FL74, producing FeLV of subgroups A, B and C, and 3201B, an FeLV nonproducer line established in this laboratory from a naturally acquired feline lymphosarcoma (W.D.H. Jr *et al.*, in preparation). The cells were maintained as suspension cultures in T-75 plastic flasks (Falcon) using McCoy's medium and 15% fetal bovine serum (FBS). Cell surface proteins were radioiodinated, solubilised, immunoprecipitated and electrophoresed as described in the legend to Fig. 1. *a–c* show polypeptides immunoprecipitated from an FL74 cell lysate with FOCMA typing serum 3192A (*a*), FeLV-neutralising serum 2934C (*b*) and control cat serum 2263A (*c*). Polypeptides immunoprecipitated from 3201B cells with feline sera 3192A, 2934C and 2263A are shown in *d, e* and *f*, respectively. ●, ¹²⁵I c.p.m.; ○, ³H-glucosamine-labelled AKR gp70.



of reactivity in the FeLV-neutralising serum could be achieved with a 70,000 dalton fraction from purified FeLV obtained by gel filtration in the presence of 6 M guanidine hydrochloride (GuHCl)¹⁶, whereas fractions containing p30, p15 and p12 had negligible effects. No absorption of reactivity in the FOCMA-specific serum was achieved with any of these fractions of virus in a parallel experiment. The above results indicate that feline LSA cells express on their surface a 70,000-dalton molecule carrying FOCMA which can be distinguished serologically from cell-surface antigens related to FeLV structural proteins.

FOCMA is also induced by FeSV infections, and evidence suggests that in this instance FOCMA may actually be encoded in the FeSV genome⁹. By gel filtration in 6 M GuHCl of proteins from mink cells nonproductively transformed by FeSV, Stephenson *et al.* detected a protein of 85,000 daltons (p85) expressing FeLV p15 and p12 and FOCMA antigens⁹. Pulse-chase experiments showed that this protein is cleaved to give a 65,000-dalton species containing FOCMA and a 25,000-dalton species containing p15 and p12. In addition, Sherr *et al.* described a phosphorylated polypeptide in pseudotype feline sarcoma virus virions (pp85) that seems to be analogous to the p85 precursor described above¹⁰. Surface molecules of FeSV-transformed nonproducer mink cells (64F3CL7) carrying FOCMA were analysed by radioiodination, immunoprecipitation and SDS-PAGE (Fig. 3). In this case, two protein species were precipitated by FOCMA antiserum, and their mobilities in gels corresponded to the intracellular p85 and p65 species described previously. Neither species was precipitated by the control cat serum (Fig. 3a). Identification of the more slowly migrating species as p85 was supported by our finding that it was precipitated with rabbit antiserum to FeLV p12 (Fig. 3b) or p15 (not shown) but was not reactive with anti-FeLV p30 (Fig. 3b). In a control experiment no protein species from similarly

labelled mink cells nonproductively transformed by Kirsten murine sarcoma virus was precipitated by the anti-FOCMA serum (Fig. 3c). These results suggest that the FOCMA-associated p85 and p65 molecules previously described in FeSV-transformed mink cells are associated with the cell membrane. It is likely that the p65 on 64F3CL7 cells is biochemically similar if not identical to the 70,000-MW species described on feline LSA cells. However, in an analysis of many primary feline LSAs we have not detected a larger species (that is, p85) containing both FOCMA and FeLV gag gene-coded antigens. This suggests that expression of a FOCMA-associated p85 species may be a unique feature of FeSV-transformed cells, and possibly of 64F3CL7 cells in particular.

The biological function of the FOCMA protein is unknown. Purification and further biochemical analysis of this protein should contribute to a better understanding of initiation and maintenance of neoplastic transformation.

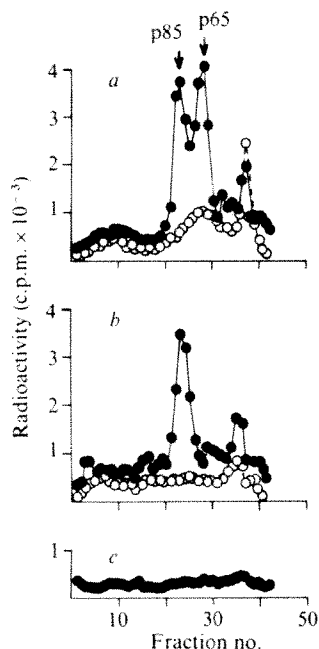
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Fig. 3 SDS-PAGE analysis of ¹²⁵I-labelled surface proteins of nonproducer mink cell clones transformed by FeSV (64F3CL7) or by the Kirsten murine sarcoma virus (64J1) precipitated from cell lysates by antibodies in cat sera and immune rabbit sera. The cells were maintained as monolayers in plastic T-75 flasks (Falcon) using Dulbecco's high glucose medium and 15% FBS. Cell surface proteins were radioiodinated, solubilised, immunoprecipitated and electrophoresed as described in the legend to Fig. 1. *a*, Polypeptides immunoprecipitated from 64F3CL7 cells with FOCMA typing serum 3192A (●) and control feline serum 2263A (○); *b*, polypeptides precipitated from this lysate with rabbit anti-FeLV p12 (●), anti-p30 (○); *c*, proteins precipitated from a lysate of 64J1 cells with 3192A serum.



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Cytogenetic mapping of the trisomic segment of chromosome 15 in murine T-cell leukaemia

A NONRANDOM cytogenetic change in the form of trisomy of chromosome 15 is associated with mouse T-cell leukaemia induced by various agents, such as Gross, Rad and Moloney leukaemia viruses, X rays and a chemical carcinogen, 7,12-dimethylbenz(a)anthracene (DMBA)¹⁻⁴. The uniformity of the chromosomal change and its apparent independence of the inducing agent suggest that it plays an important and perhaps decisive part in the neoplastic transformation of the mouse T lymphocyte. During our studies of the role of chromosome 15 and related factors in lymphoma development, we have investigated whether any particular region of the chromosome needs to be duplicated. Using DMBA to induce T-cell leukaemias in

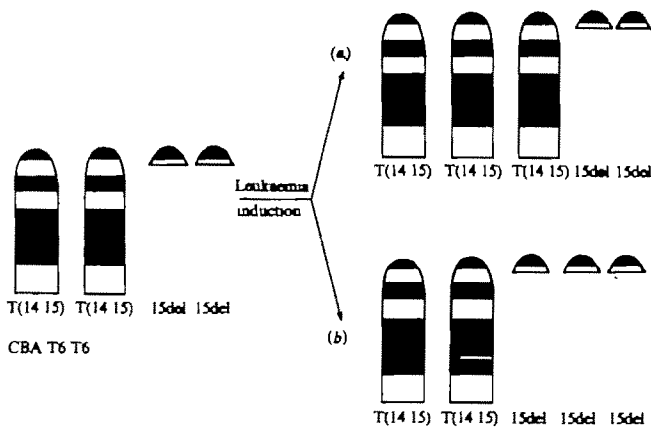


Fig. 1 Schematic illustration of two alternatives (a and b) for chromosome 15 trisomic T-cell leukaemias in CBAT6T6 mice.

mice carrying chromosome 15 translocations, we have found, and now report that the duplicated chromosome segment is located distal to the T6 breakpoint of chromosome 15.

T-cell leukaemias were induced by feeding DMBA to female and male CBAT6T6 mice (Weizmann Institute) as described elsewhere⁵. Overt leukaemias were transplanted subcutaneously to CBA recipients of the opposite sex. The chromosomes of leukaemic cells of the first passage generation were analysed by G banding. Banded metaphase plates were prepared from subcutaneous tumours, spleens and bone marrow by a slight modification of Wang and Fedoroff's procedure⁶. Magnified photographs were used for karyotype analysis, and trisomic chromosomes were also identified under the microscope in metaphase plates with high banding quality. Chromosome identification followed the nomenclature of the Committee of Genetic Standardization⁷. Because all leukaemic cells originated from donor CBAT6T6 mice, they could be distinguished easily from host cells by the double criteria of the sex chromosome and the T6T6 markers. The CBAT6T6 mouse carries the reciprocal translocation T(14;15)6 (ref. 8). Chromosome 15-trisomic T-cell leukaemias were expected to conform to one of the two patterns illustrated schematically in Fig. 1.

Chromosome counts of six tumours are summarised in Fig. 2. Five out of six leukaemias were aneuploid; tumours with a modal number of 41 chromosomes and tumours with a modal number of 42 and 40 + 1 bi-armed (total 42) chromosomes. Only one tumour (mice 2 and 3) had a modal number of 40 chromosomes. Table 1 shows the results of banding analysis. All five aneuploid leukaemias with a modal number of 41 and 42

Fig. 2 Chromosome (Chr.) constitution of cells originating from subcutaneous (s.c.) tumours, spleens and bone marrow of CBA mice inoculated with DMBA-induced CBAT6T6 leukaemic cells. * 1 Bi-armed chromosome.

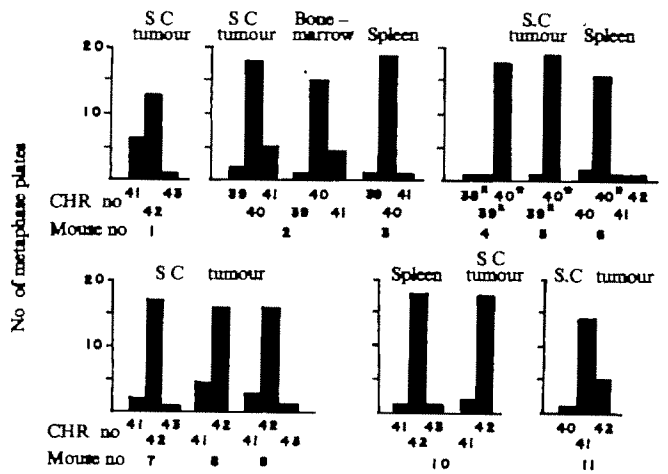


Table 1 G-banding analysis of leukaemic cells with aneuploid chromosome constitution			
Mouse no.	Modal chromosome no.	No. of plates analysed by banding	Trisomy of chromosome†
1	42	14+8*	T(14;15)6
2	40	20+10*	
5	40+1B	15+15*	T(14;15)6
9	42	10+5*	T(14;15)6
10	42	12+4*	T(14;15)6
11	41	10+8*	T(14;15)6

B, bi-armed. The bi-armed chromosome was formed by centromeric (Robertsonian) fusion between one of chromosomes T(14;15)6 and chromosome 12.

* Karyotyped from magnified photos.

† The second trisomy involved mostly chromosomes 2, 6 and 19.

chromosomes had a trisomy T(14;15)6 (Fig. 3). The second trisomy of tumours with a modal number of 42 chromosomes was distributed randomly, involving mostly chromosomes 2, 6 and 19. The banding pattern of the single tumour with a modal number of 40 chromosomes was indistinguishable from a diploid CBAT6T6 cell.

These results show that the gene(s) that tend to be duplicated during DMBA leukaemogenesis are located distal to the T6 breakpoint of chromosome 15. The consistency of the change further emphasises the specific role of genes associated with

Fig. 3 G-banded karyotype and metaphase plate of a leukaemic cell originating from a female CBAT6T6 mouse and inoculated s.c. into a male CBA mouse. Note the presence of trisomy T(14;15)6 (arrows). The second trisomy involved chromosome 6.



chromosome 15 in T-cell leukaemia. However, the occurrence of a diploid leukaemia in one tumour shows that the change is not exclusive. This agrees with previous findings on AKR and the RadLV leukaemias^{1,2}. In possible analogy with the present work, a specific chromosome aberration affecting one chromosome 14 was found in human B-cell derived neoplasias³, suggesting that certain chromosome segments carry genes that are critical to the neoplastic growth of given target cells.

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An inducible gene involved in commitment of lymphocytes to transform

CONCAVALIN A (Con A) stimulates nondividing lymphocytes (in G_0) to transform and enter the division cycle¹. Within 3 h a significant proportion of the cells become committed to transform and do so after the removal of Con A (ref. 2). When the translation of mRNA into protein is inhibited (by low concentrations of anisomycin) lymphocyte transformation is also blocked³. Anisomycin has proved particularly useful in studying lymphocyte transformation because its effect is reversible: in the presence of Con A it holds the cells in G_0 , apparently without adverse effects, and the cells transform following its removal³. Using anisomycin it has been shown that Con A induces the synthesis of factor(s) which commit lymphocytes to transform³. I have examined the stability and nature of the 'transformation signal', again exploiting the reversible effect of anisomycin, and report here that on binding to the surface of nondividing lymphocytes, Con A induces the transcription of a short-lived mRNA. This mRNA encodes a 'transformation factor' which, when translated, commits lymphocytes to enter the division cycle; extracellular Con A is no longer required.

Spleen cells from BALB/c mice (6–10-week-old males) were cultured in RPMI medium (see legend to Table 1). Transformation was measured by the incorporation of tritiated thymidine ($^3\text{H-TdR}$) into cellular DNA over 48–50 h; the stimulation index was calculated as the ratio of c.p.m. in Con A-treated cultures to c.p.m. in control cultures. The mitogenic response to Con A peaked at $1 \mu\text{g ml}^{-1}$ and this concentration was used routinely. Anisomycin (10 ng ml^{-1}) was used to hold Con A-treated cells in G_0 for the first 24 h in culture, after which extracellular Con A was removed using α -methyl-D-

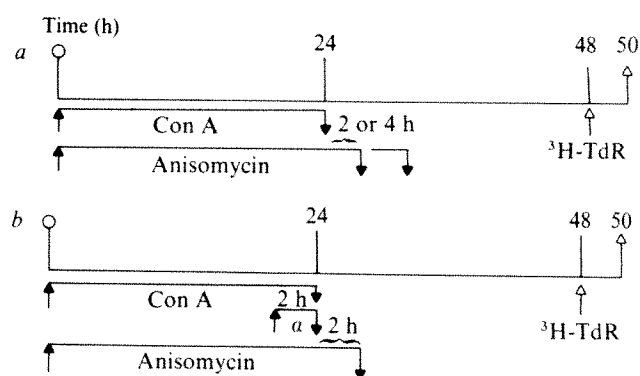


Fig. 1 Plan of experimental procedures described in the text. *a*, The $t_{1/2}$ of the transformation signal was estimated by treating BALB/c spleen cells with Con A ($1 \mu\text{g ml}^{-1}$) plus anisomycin (10 ng ml^{-1}) for 24 h. Con A was removed at 24 h using α -methyl-D-mannoside (50 mM), and anisomycin was continued for a further 2 or 4 h before it also was removed. The cells were then cultured in normal medium and $^3\text{H-TdR}$ was added at 48 h for 2 h to assess DNA synthesis. The cells were collected at 50 h. *b*, The possibility that the transformation signal might be mRNA was examined by culturing BALB/c spleen cells with Con A ($1 \mu\text{g ml}^{-1}$) plus anisomycin (10 ng ml^{-1}) for 24 h. At 22 h α -amanitin ($10 \mu\text{g ml}^{-1}$) was added to inhibit mRNA transcription for 2 h and mRNA translation was then inhibited by anisomycin for a further 2 h after the removal of Con A plus α -amanitin. The cells were subsequently cultured and labelled as above. α = α -amanitin.

mannoside³. Additional experimental procedures are summarised in Fig. 1.

The stability of the transformation signal was estimated by continuing anisomycin for various periods after the removal of Con A. The cells recovered and transformed when anisomycin was continued for 2 h after Con A removal but when it was continued for 4 h after Con A removal cell transformation fell by 60% (Table 1). This indicates a half life ($t_{1/2}$) of approximately 3–4 h for the transformation signal (in the presence of anisomycin). This relatively short $t_{1/2}$ suggests that it must be continually expressed in Con A-treated cells held in G_0 for 24 h (by anisomycin) because the cells transform following the simultaneous removal of mitogen plus inhibitor³. Continuous production of the transformation factor presumably depends on the continued presence of Con A, as the ability of the cells to transform fell rapidly 2 h after the removal of Con A (Table 1).

α -Amanitin, a selective inhibitor of mRNA transcription^{4,5}, was used to examine the possibility that the transformation signal might be a species of mRNA transcribed in response to Con A. If the signal is Con A-dependent mRNA, with a $t_{1/2}$ of 3–4 h, it follows that cell transformation should decline markedly when mRNA transcription (in the presence of Con A) and translation (in the absence of Con A) are inhibited sequentially for 2 h each. Again, spleen cells were cultured with Con A plus anisomycin for 24 h. α -Amanitin ($10 \mu\text{g ml}^{-1}$) was added to the cultures at 22 h and removed at 24 h, together with the Con A (Fig. 1*b*). Anisomycin was continued for a further 2 h before it also was removed. Experimental controls showed that continued exposure to α -amanitin blocked transformation, whereas cells recovered and transformed when exposed to either α -amanitin at 22–24 h or continued anisomycin for 2 h after Con A removal (Table 2). However, transformation fell by 50% in those cells treated with α -amanitin 2 h before and anisomycin 2 h after the removal of Con A. In other words, the sequential inhibition of mRNA transcription and translation for a total of 4 h resulted in a 50% drop in cell transformation, consistent with the transformation signal being mRNA.

Interpretation of these results clearly depends on the actions of the two antibiotics α -amanitin and anisomycin. In cell-free systems, each has been shown to be highly selective: anisomycin blocks peptidyl transferase activity on 80S ribosomes⁶ and α -amanitin inhibits RNA polymerase II^{4,5} (mRNA synthesis). At very high concentrations ($\geq 200 \mu\text{g ml}^{-1}$) α -amanitin also

Table 1 Recovery and transformation after prolonged anisomycin inhibition for 1, 2 and 4 h after removal of Con A (see Fig. 1a)

Treatment	³ H-TdR incorporation (c.p.m.)	Stimulation index
Untreated control	2,645	—
Con A throughout	48,273	18
I Con A + anisomycin throughout	3,267	1.2
Con A removed at 24 h	44,842	17
Con A + anisomycin, removed at 24 h	39,287	15
II Con A + anisomycin, removed at 25 h	48,346	18
Con A + anisomycin, removed at 26 h	42,375	16
Con A + anisomycin, removed at 28 h	17,290	6.5

BALB/c spleen cells exposed to Con A ($1 \mu\text{g ml}^{-1}$) and anisomycin (1 ng ml^{-1}): the $t_{1/2}$ of the transformation factor was determined by continuing anisomycin inhibition for various periods after removal of Con A at 24 h. Washed spleen cells from a single mouse were diluted to $5 \times 10^6 \text{ ml}^{-1}$ with RPMI plus 100 IU ml^{-1} penicillin and 100 $\mu\text{g ml}^{-1}$ streptomycin. Aliquots (100 μl) were dispensed into flat-bottomed wells of a microtitre tray and cultured in a humidified atmosphere of 10% CO_2 in air at 37°C . After removal of Con A (group II) the medium was replaced with Con A-free medium plus α -methyl-D-mannoside (50 mM) to displace any cell-bound Con A (ref. 3). At 25 h the medium was replaced with normal medium. Anisomycin (10 ng ml^{-1}) was added to the replacement media for 1, 2 or 4 h after the removal of Con A; thereafter, it was replaced with normal medium. Transformation was assessed by measuring DNA synthesis using standard techniques after labelling each culture with 2 μCi [$6\text{-}^3\text{H}$]thymidine (specific activity 5 Ci mmol^{-1}) for 2 h at 48–50 h. Each value represents the mean of four cultures.

inhibits RNA polymerase III⁷ (tRNA and 5S RNA synthesis). Intact cells are reputed by some to be impermeable to α -amanitin and this seems to be true for rat hepatoma cells⁸. However, this is not a generalised phenomenon and normal rat liver cells *in vivo*⁹, adrenal cortical cells⁹ and lymphocytes *in vitro* (this work) are all sensitive to α -amanitin at concentrations known to block mRNA synthesis selectively. α -Amanitin may also indirectly affect rRNA synthesis which seems to depend on

Table 2 Recovery and transformation after the sequential inhibition of mRNA transcription (by α -amanitin) and translation (by anisomycin) for 2 h each (see Fig. 1b)

Treatment	³ H-TdR incorporation (c.p.m.)	Stimulation index
Untreated control	550	—
Con A	52,000	95
I Con A + α -amanitin throughout	480	0.9
Con A + anisomycin throughout	4,108	8
Con A	34,708	63
Con A + α -amanitin for 2 h, at 22–24 h	29,261	53
Con A + anisomycin continued for 2 h after Con A removal	28,100	51
II Con A + α -amanitin (2 h at 22–24 h) + anisomycin (continued for 2 h after removal of Con A + α -amanitin)	13,307	24

BALB/c spleen cells cultured and labelled as described in the legend to Table 1. Group I, Con A present throughout, group II, Con A removed at 24 h. Anisomycin (10 ng ml^{-1}) and α -amanitin ($10 \mu\text{g ml}^{-1}$) were added to the culture medium as indicated. Each value represents the mean of four cultures.

mRNA with a $t_{1/2}$ of about 20 min (see ref. 10, in which actinomycin D, rather than α -amanitin, was used). If rRNA synthesis in lymphocytes is similarly affected by α -amanitin the effect must be reversible, as inhibition for 2 h is reversible (Table 2). It follows that the present results with α -amanitin cannot be attributed to an indirect effect on rRNA synthesis.

Using α -amanitin and anisomycin as inhibitors of mRNA transcription and translation respectively it has been shown that (1) the transformation factor in lymphocytes seems to be coded for by mRNA; (2) the mRNA has a $t_{1/2}$ of 3–4 h; (3) extracellular Con A is necessary for its continuous production when cell transformation is blocked by anisomycin. The effect of anisomycin is reversible and when mRNA translation is allowed to proceed normally (within 2 h of Con A removal), the cells transform independently of extracellular Con A. On the basis of these observations it is postulated that Con A induces the transcription of a new species of mRNA(s) in lymphocytes. When this mRNA is translated the newly synthesised protein takes over and commits the cells to enter the division cycle; extracellular Con A is no longer required.

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Release of vasoactive intestinal polypeptide in mast cells by histamine liberators

VASOACTIVE INTESTINAL POLYPEPTIDE (VIP), originally isolated from porcine duodenum¹, is a potent relaxant of vascular and nonvascular smooth muscle², and a stimulant of adenylate cyclase activity^{3,4}. The peptide has been localised in endocrine cells in the gastrointestinal tract and pancreatic islets^{5,6}, as well as in nervous structures in these and other organs^{7–9}, the peripheral autonomic nervous system and selected areas of the brain^{8–11}. We report here immunological evidence that VIP, or a closely related peptide, occurs in mast cells, from which it can be released by compound 48/80 or by the Ca^{2+} ionophore A23187.

Rat peritoneal mast cells were collected from the peritoneal cavities of rats. Under ether anaesthesia, the rats were injected intraperitoneally with 12 ml of Sorensen phosphate buffer, pH 7.0. The rats were killed with ether 5–10 min later, their abdomens were opened and the peritoneal fluid removed and centrifuged at 400g for 8 min. The cell pellet, resuspended in 2–3 ml of buffer per rat, contained approximately 10^6 mast cells (10% of the total cell population). Aliquots of washed cell suspensions were incubated for 15 min at 37°C with 0.1 μg per ml of compound 48/80 (Burroughs-Wellcome) or 1 μg per ml of Ca^{2+} ionophore A23187 (Lilly). After incubation the media were separated from the cells by centrifugation at 400g for 8 min. The supernatants were decanted and then assayed for VIP immunoreactivity by a specific radioimmunoassay¹² sensitive to 10 pg per ml, and for histamine by the fluorometric method of Shore¹³. The amounts of the peptide and amine released were expressed as per cent of their respective total

Table 1 VIP and histamine in rat peritoneal mast cells

	VIP	Histamine
Total content (ng ml) ⁻¹	3.6 ± 0.8 (6)	689.3 ± 3 (6)
Release (% of total)		
48/80	45 ± 10 (5)	48 ± 4 (5)
A23187	50 ± 10 (4)	67 ± 6 (4)

Figures are means ± s.e.m.; numbers of determinations are in parentheses.

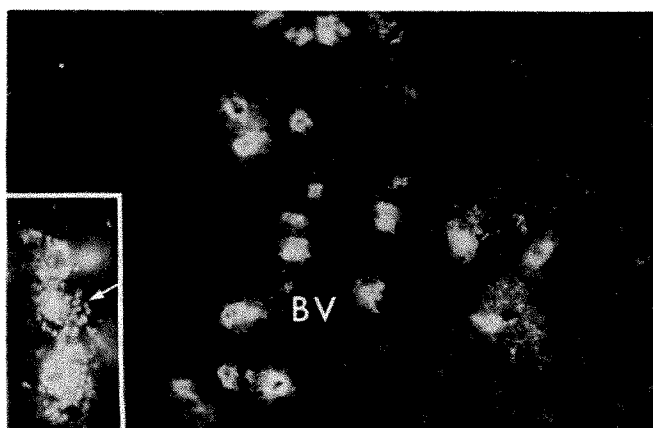
content, measured after disrupting the cells by boiling in water for 3 min.

The mean VIP content in suspensions of rat peritoneal mast cells (3.6 ± 0.8 ng per ml or 1.4 ng per 10⁶ mast cells) was a fraction of their histamine content (689.3 ± 3 ng per ml); 1 ml contained approximately 4 × 10⁵ mast cells. Incubation of the cells with compound 48/80 or with A23187 provoked release of the peptide in proportions similar to those of the associated histamine release (Table 1).

For immunohistochemical studies, VIP antibodies were raised in rabbits against porcine VIP (GIH Laboratory, Stockholm), and included the same antibody (89N) used for radioimmunoassay, and two others (TR₁ and TR₂). Samples of lung and small intestine were obtained from adult and newborn Wistar rats and from mice, and were processed for immunohistochemistry according to the method of Pearse and Polak¹⁴. The samples were quenched in melting Arcton 22, freeze-dried, vapour-fixed in recrystallised benzoquinone at 60 °C for 3 h and embedded in paraffin; sections were cut at 5–7 µm. Pellets of rat peritoneal mast cells were processed for immunohistochemistry in the same way. The indirect immunofluorescence technique (24–48 h incubation at 4 °C) was carried out using the three VIP antibodies (dilutions 1:5 to 1:100), and antibodies against porcine secretin, synthetic human G-17 gastrin and porcine glucagon. As a second layer, fluorescein-labelled goat anti-rabbit IgG was used. The following control procedures were carried out: (1) incubation with VIP antisera (1:5) to which pure VIP (60 µmol per ml) had been added; (2) the first layer was replaced by non-immunised rabbit serum or phosphate buffer; (3) incubation with the second layer only. Following examination under the fluorescence microscope the sections were stained with toluidine blue (pH 5) to identify and confirm the presence of mast cells.

Sections of rat and mouse lungs incubated with VIP antisera (up to 1:100 dilutions) showed strong positive fluorescence in mast cells in perivascular, peribronchial and interstitial locations (Fig. 1). Examination at higher magnification revealed that VIP was localised within mast-cell granules (Fig. 1 inset). Mast cells

Fig. 1 Many strongly fluorescent, VIP-immunoreactive mast cells in rat lung. Mast cells are scattered in the connective tissue, but some are concentrated along the wall of a blood vessel (BV). (Indirect immunofluorescence method, VIP antibody TR₂, 1:50, ×400.) Inset, higher magnification (×900) of mast cells showing VIP immunoreactivity confined to cytoplasmic granules (arrow).



in sections of small intestine and in cell preparations from peritoneal lavage also showed strong VIP immunoreactivity. No fluorescence was observed with VIP-treated VIP antisera, or with the antisera against the other peptides. Mast cells were cross-identified in sections stained with toluidine blue; the cells contained typical metachromatic granules and their size, appearance and distribution were identical to those of VIP-immunoreactive cells.

The demonstration of immunoreactive VIP in mast cells from different locations from two mammalian species, and the release of the peptide by histamine releasers suggest interesting functional implications. The mast cell is the principal target cell in immediate hypersensitivity, playing a key part in anaphylaxis, extrinsic (allergic) asthma, hay fever and other IgE-dependent immunological responses¹⁵. The release of VIP by the ionophore A23187 is of special interest as this agent releases histamine by a mechanism similar to that of anaphylaxis¹⁶. As VIP stimulates the production of cyclic AMP in the lung and other tissues^{3,4}, and as agents which promote cyclic AMP accumulation in the lung also inhibit mediator release¹⁷, VIP could have an important modulating influence on the release of histamine and other mediators from mast cells.

Further, VIP may, when discharged from pulmonary mast cells, exert its relaxant actions on bronchial and vascular smooth muscle², providing another level of modulation of airway and vascular responses by the peptide.

VIP immunoreactivity has been reported in peptide extracts of normal porcine lungs¹⁸. The evidence presented here of VIP immunofluorescence in pulmonary mast cells provides an anatomical basis for that finding. Finally, mast cells are widely distributed in connective tissues of various organs of the body and are generally concentrated along the blood vessels. VIP in mast cells is therefore in a position to influence local or regional blood flow, in normal or pathological conditions.

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Are dopamine receptors accessible to intracellularly applied agonist?

THERE is much evidence that receptors to neurotransmitters are generally on the outer surface of cell membranes¹⁻³. The experiments reported here raise the possibility that acinar cells of cockroach salivary glands may be activated intracellularly as well as by extracellularly applied agonists.

Studies were performed using the isolated innervated salivary gland of *Nauphoeta cinerea* (Olivier) bathed in flowing solution (2 ml min⁻¹) containing (mM) NaCl, 160; KCl, 1; CaCl₂, 5; Tris-HCl pH 7.6 buffer, 5 and mounted as previously described⁴. Cells were impaled with microelectrodes containing agonists in concentrations of up to 1.0 M dissolved in either potassium acetate or KCl. The electrodes were used not only for intracellular recording but also for ejecting the agonist by means of current pulses. The reservoir ducts were drawn into a small suction electrode to allow stimulation of the salivary nerve. Figure 1a shows two successive responses of an acinar cell, N to a single nerve stimulus and D to the application of a current pulse to the intracellular electrode equivalent to a charge of 40 nC. The responses can be seen to be very similar. In Fig. 1b the response to a burst of nerve stimuli, N, is followed by three responses to increasing ionophoretic charges and it can be seen that there is a graded relationship between amplitude of response and charge.

Intracellular applications of dopamine were made to about 100 cells and responses were obtained from about two-thirds of these. In a few experiments responses were also obtained from intracellular applications of adrenaline, noradrenaline and 5-hydroxytryptamine which, like dopamine, have also been shown to be effective when applied extracellularly⁵.

Two possible explanations for these responses are (1) receptors are activated intracellularly by the ionophoretically applied agonists or (2) the receptors on the outer membrane surface are activated by agonist which has leaked through the membrane. Attempts to distinguish between (1) and (2) have so far been inconclusive. In one experiment we compared the sensitivity of a series of cells to extra- and intracellular application from the same ionophoretic pipette. The cell was first impaled with a separate recording electrode, the ionophoretic pipette was brought as close as possible to the outer surface of the acinus and a response to a pulse of dopamine was elicited. An attempt was

Fig. 1 a, Lower trace, voltage; upper trace, current passed through recording electrode. N, intracellularly recorded response to a stimulus applied to the salivary nerve; D, response to a dopamine pulse ejected by a 100 nA current for 0.4 s. Downward deflection corresponds to hyperpolarisation. The electrode contained 0.7 M dopamine and 1 M potassium acetate. b, Intracellular response, N, to a burst of three nerve stimuli followed by responses to three pulses of dopamine ejected from the intracellular electrode by currents of 100 nA for durations of 0.2, 0.4 and 0.6 s respectively. The electrode contained 0.3 M dopamine and 1 M potassium acetate.

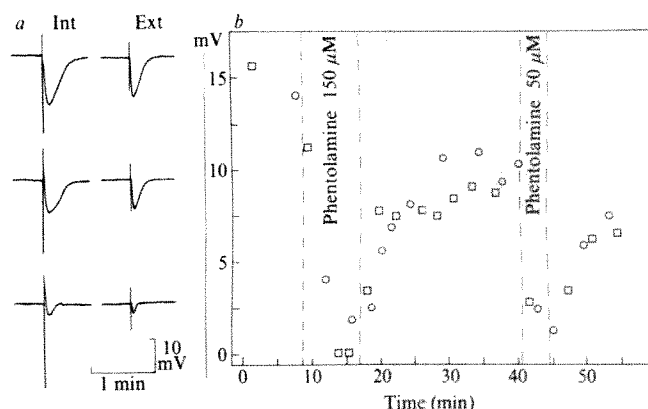
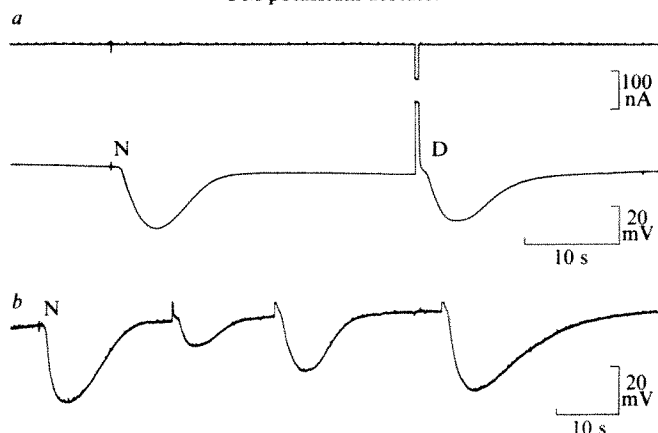


Fig. 2 Effect of phentolamine on intracellularly recorded responses from the same cell. a, Int, pulses of dopamine ejected from the intracellular electrode (100 nA for 0.4 s); Ext, pulses of dopamine ejected from an extracellular pipette (200 nA for 0.08 s). Both electrode and pipette contained 0.5 M dopamine and 3 M potassium acetate. Top records, control; middle records, during recovery from a short exposure to 150 μ M phentolamine; bottom records, in 25 μ M phentolamine. b, part of time course of experiment from which records in a were taken. $\circ$, Intracellularly applied dopamine; $\square$, extracellularly applied dopamine.

then made to insert the ionophoretic pipette. The initial recording electrode was usually dislodged in the process but this did not matter because if the cell was successfully impaled with the ionophoretic pipette it could also be used for recording. Little difference was found between the maximum sensitivities with the two different methods of application. For an electrode containing 50 mM dopamine in 3 M potassium acetate the largest value for the ratio of response to ionophoretic charge was 3 mV nC⁻¹ for intracellular and 2.3 mV nC⁻¹ for extracellular application. (If it is assumed that half the current is carried by cations and that the transport number of the dopamine ion is the same as that of K⁺, 1 nC would correspond to the ejection of about 10⁻¹⁶ mol; this would give rise to an internal concentration of about 10⁻⁷ M if the dopamine was uniformly distributed throughout an acinus of the usual diameter of about 100 μ m.) This result might be thought to argue against the leakage hypothesis, (2), but the result of a second type of experiment was found to be consistent with it. Cells were impaled with one ionophoretic pipette for intracellular recording and dopamine application and a second similar pipette was placed close to the acinar surface for extracellular dopamine application. After matching responses were obtained, phentolamine⁵, which is known to antagonise extracellularly applied dopamine⁵, was added to the flowing bathing solution. Responses to both intracellular and extracellular dopamine were reduced to the same extent (Fig. 2); thus the leakage hypothesis cannot be rejected. However, as phentolamine may be able to leak into the acinar cells, the possibility that the receptors might be accessible from both sides of the membrane cannot be excluded.

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Ionophoretic model for Na-Ca counter transport

IN most mammalian cells the cytosolic concentration of free ionised Ca^{2+} is thought to approximate 10^{-6} – 10^{-7} M, whereas the extracellular Ca^{2+} concentration is close to 10^{-3} M (ref. 1). The efflux of Ca^{2+} across the cell membrane, which takes place against an electrochemical gradient, is apparently mediated either by a Ca^{2+} -stimulated ATPase² or by a Na-Ca counter transport process. In the latter process, the entry of Na^+ into the cell, which is a passive event driven by the electrochemical gradient of Na^+ across the plasma membrane, is thought to be coupled with the uphill outwards transport of Ca^{2+} (ref. 3). Although a possible modulatory effect of ATP on such a process has been suggested, it is generally assumed that the Na-Ca counter transport mechanism does not consume ATP (discussed in ref. 4). Because several passive ionic movements (such as the influx of Na^+ or Ca^{2+} into the cell) could be mediated by native ionophoretic molecules, we have explored whether it was possible, using an artificial system for the study of ionophoresis, to reproduce the major characteristics of the Na-Ca counter transport process. We report here our success.

A CCl_4 organic phase (3.2 ml) containing Br-X537A (1.4 mM, Roche) was placed in the bottom of a Pressman cell⁵. Ionophore Br-X537A was used because it transports both monovalent and divalent cations⁶. The composition of the supernatant aqueous phases (1.6 ml) which was placed in each chamber of the Pressman cell is given in the legend of Fig. 1.

At the end of the experiments—after 24 h—the immiscible organic phase contained only trace amounts of ^{22}Na , representing no more than $0.22 \pm 0.05\%$ of the total radioactivity in the system. The ^{45}Ca content of the final immiscible phase was greater. Relative to the total amount of radioactivity present in the system and with 4 mM Ca^{2+} in each chamber, it averaged $4.5 \pm 0.7\%$ ($n=13$) and $11.8 \pm 1.0\%$ ($n=8$) in the presence of Na^+ (121 mM in both chambers) and the absence of Na^+ , respectively. These values correspond to apparent calcium concentrations, in the immiscible phase of 0.18 ± 0.03 and 0.47 ± 0.04 mM (as distinct from 2.4 ± 0.9 μM in the absence of X537A).

In the absence of ionophore, no significant amount of ^{22}Na ever seemed to be translocated. Relative to the total amount of ^{45}Ca present in the system, the transfer rate for ^{45}Ca averaged no more than $0.21 \pm 0.03\%$ per h.

In the presence of Br-X537A but in the absence of ionic gradient, that is when both aqueous phases had the same ionic concentration at time zero, ^{45}Ca (which was added to only one of the chambers) moved towards the other chamber, and eventually the radioactivity was equally distributed among both chambers (Fig. 1, left). At the same Ca^{2+} concentration (4 mM), the initial rate at which the radioactivity moved from one chamber to the other was greater in the absence than in the presence of Na^+ (120 mM). The latter observation is consistent with the fact that Na^+ decreases the ability of the ionophore to translocate ^{45}Ca into an organic immiscible phase (our unpublished observation). When 4 mM Ca^{2+} and 120 mM Na^+ were present in both chambers, ^{22}Na was transferred relatively slowly (approximately 1.0% of the total radioactivity transferred per h) so that, even after 24 h, the partition equilibrium for ^{22}Na was not reached.

The pattern for ^{45}Ca translocation was different when an ionic gradient was imposed on the system at time zero. When 4 mM Ca^{2+} was present in both chambers, but Na^+ was distributed asymmetrically (120 mM out (O) and 6 mM in (I)), most of the ^{45}Ca initially added to the I compartment was transferred to the O compartment. After 24 h, more than 80% of the total radioactivity was recovered in the O chamber. This occurred independently of any electrical gradient, also being observed

when the two chambers were connected by an agar-agar bridge (4%, w/v, in Hartmann's solution Na^+ 158 mM, K^+ 5 mM, Ca^{2+} 2 mM, Cl^- 139 mM, $\text{CH}_3-\text{CHOH}-\text{CO}_2^-$ 28 mM). When, in addition to the Na^+ gradient (120 mM O and 6 mM I), a Ca^{2+} gradient (0.2 mM I and 4 mM O) was also introduced, up to 90% of the ^{45}Ca initially present in the I compartment was transferred to the O compartment. A dramatic uphill translocation of $^{45}\text{Ca}^{2+}$ (I $\rightarrow$ O) also occurred when only a trace of $^{45}\text{Ca}^{2+}$ (1.0 μM) and no $^{40}\text{Ca}^{2+}$ was added to the I chamber (data not shown). These observations indicate that ^{45}Ca can move against the Ca^{2+} gradient. The initial rate of ^{45}Ca transfer was much greater when the Ca^{2+} gradient was superimposed on to

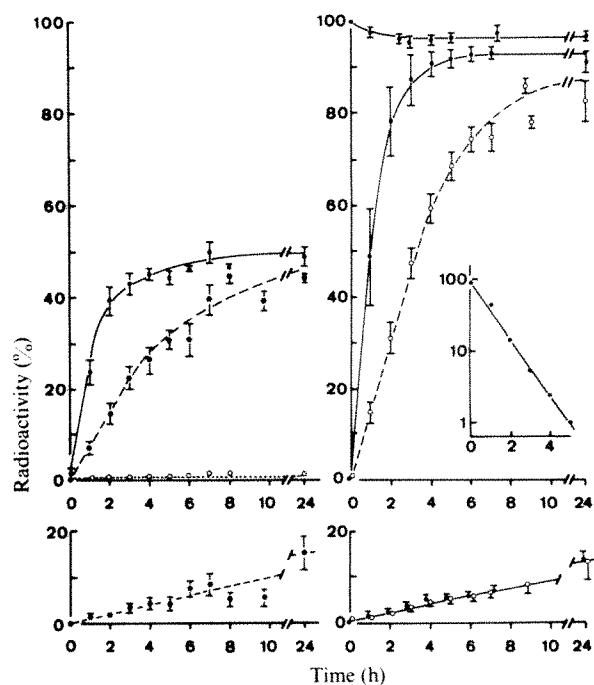


Fig. 1 Transfer of ^{45}Ca (upper graphs) and ^{22}Na (lower graphs) in a Pressman cell, across an immiscible organic phase containing Br-X537A. The aqueous phases consisted of a Hepes solution (25 mM) buffered to pH 7.0 with either NaOH or $\text{Ca}(\text{OH})_2$ and containing, as required, CaCl_2 , NaCl and trace amounts of $^{45}\text{CaCl}_2$ (0.5–1.0 $\mu\text{Ci ml}^{-1}$) or $^{22}\text{NaCl}$ (0.25 $\mu\text{Ci ml}^{-1}$). The organic phase was stirred continuously (about 120 r.p.m.) with a magnetic bar, the experiments being carried out at room temperature (about 22 °C). At the onset of the experiments and at timed intervals thereafter, a sample of the aqueous phase (0.05 ml) was removed from each chamber and examined for its radioactive content by liquid scintillation counting. The observed radioactivity was corrected for the change in volume due to the sampling procedure. Evaporation was minimised by the use of a glass cover and was corrected for by expressing individual data as a percentage of the total radioactivity recovered at each sampling time in both the I and O chambers. In the left panels, both aqueous phases contained Ca^{2+} (4 mM) in the presence (★) or absence (●) of Na^+ (120 mM). The control data for ^{45}Ca translocation obtained in the absence of Br-X537A are also shown (○). In the right-hand graphs, a Na^+ gradient (120 mM O, and 6 mM I) was always used, either the same Ca^{2+} concentration (4 mM) being initially present in both aqueous phases (○) or a Ca^{2+} gradient (0–0.2 mM I, and 4 mM O) being superimposed on the Na^+ gradient (●). The data for ^{45}Ca always refer to the radioactivity found in the O compartment, whether ^{45}Ca was initially located in the I (ascending curves) or O (upper flat curve) chamber. The data for ^{22}Na always refer to the radioactivity found in the I compartment, the tracer being in all cases initially located in the O chamber. In the inset (upper right graph), the data for ^{45}Ca transfer (●) are shown in a semi-logarithmic plot, the difference between equilibrium and observed values for ^{45}Ca being ranged on a logarithmic scale. Mean values ($\pm$ s.e.m.) refer to 6–12 individual experiments.

the Na^+ gradient than when only the Na^+ gradient was initially present (Fig. 1, right). This difference is attributable to the much lower initial Ca^{2+} concentration (0.2 mM compared with 4.0 mM) in the I compartment, so that much more radioactivity moved from I to O even if the same total Ca^{2+} was translocated in the same direction. Using a comparable experimental design (that is Na^+ 120 mM O and 6 mM I; Ca^{2+} nil I and 4 mM O) but with ^{45}Ca initially located in the O chamber, about 95% of radioactivity remained in the O chamber, even after 24 h of incubation. In all these experiments, ^{22}Na (which was initially located in the O chamber) moved in the I chamber at a rate comparable with that seen in the absence of Na^+ gradient (Fig. 1, lower graphs). Less than 15% of the total ^{22}Na was transferred from the O to the I chamber after 24 h of incubation.

The true concentration of Na^+ and Ca^{2+} in the two chambers was also measured. In the absence of ionophore, Na^+ and Ca^{2+} concentrations remained close to their respective initial values, whatever the experimental conditions (data not shown). In the presence of the ionophore Br-X537A and with a Na^+ gradient (Na^+ 120 mM O, and 6 mM I; Ca^{2+} 4 mM on both sides), the Ca^{2+} concentration progressively decreased in the I compartment and concomitantly increased in the O compartment at a mean rate of 0.48 ± 0.02 mM per h, so that 96% of the total amount of Ca^{2+} was present in the O chamber at the 8th h. In the presence of both Na^+ and Ca^{2+} gradient (Na^+ 120 mM O and 6 mM I; Ca^{2+} 4 mM O and nil I), no significant translocation of Ca^{2+} was observed, the concentration of Ca^{2+} plateauing at 4.06 ± 0.04 mM in the O chamber and no detectable amount of Ca^{2+} (less than 0.1 mM) appearing in the I chamber, even after 24 h. In both types of experiment, the Na^+ concentration slightly decreased in the O chamber and concomitantly increased in the I chamber at a rate not exceeding 0.8 ± 0.3 mM per h. Even at the 24th h, the concentration of Na^+ remained six to seven times higher in the O than in the I chamber.

The experimental condition which mimics more closely the situation encountered in most mammalian cells corresponds to the following distribution: in the O chamber (that is the extracellular fluid) Na^+ 120 mM and Ca^{2+} 4 mM; in the I chamber (that is the intracellular compartment) Na^+ 6 mM and $\text{Ca}^{2+} \leq 0.2$ mM. In such conditions the initial rate of transfer of radioactivity averaged 0.9% per h for $^{22}\text{Na}(\text{O} \rightarrow \text{I})$ and at least 88.0% per h for $^{45}\text{Ca}(\text{I} \rightarrow \text{O})$; see inset Fig. 1). These values correspond to cationic fluxes of 1.1 mM Na^+ per h and 0.2 mM Ca^{2+} per h. In living cells, three to four sodium ions are thought to enter the cell for each Ca^{2+} ion extruded by the Na-Ca counter transport process⁷.

In conclusion, Br-X537A can represent a model for the Na-Ca counter transport process. Our results show that a Na^+ gradient can be sufficient to maintain a Ca^{2+} gradient and to insure the transport of Ca^{2+} against such a gradient by ionophoresis.

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Potassium channels in the apical membrane of the toad gallbladder

It has been demonstrated that the apical and the basolateral cell membrane of the gallbladder have a high K^+ permeability^{1,2} and that the electrochemical potential for potassium in the cytosol is about 50 mV more positive than that in the bathing solutions³. Because of the existence of an $(\text{Na}^+, \text{K}^+)\text{ATPase}$ ⁴ which is located on the basal lateral surface of the epithelial cells⁵, it is possible that K^+ is actively transported in the cells at the serosal side and partially leaves the cellular compartment through the apical membrane under the influence of its electrochemical gradient. In the present study we have investigated this hypothesis by analysis of the current fluctuations recorded in voltage-clamp conditions. This method has been shown to be very useful for the investigation of the kinetic properties of ion transport in such different biological tissues as nerve, postsynaptic membranes^{6,7} and epithelia⁸. We have found K^+ -selective channels which open and close randomly in the apical membrane of the toad gallbladder. However, this study did not reveal the presence of fluctuating ionic channels in the serosal membrane.

The gallbladder epithelium of the Mexican toad *Bufo marinus* was mounted in a modified Ussing chamber with a free surface of 0.16 cm^2 . With Na Ringer's solutions (115 mM NaCl, 1 mM CaCl_2 , 1 mM MgCl_2 , 1.08 mM K_2HPO_4 and 0.36 mM KH_2PO_4) on both sides, the transepithelial resistance was $212 \pm 15 \Omega \text{ cm}^2$ ($n = 79$) and the transepithelial potential was $0.44 \pm 0.16 \text{ mV}$ ($n = 34$), serosa positive. Strong amplification of this potential reveals that it is not constant but fluctuates around its macroscopic mean value. These voltage fluctuations were clamped to zero with a low-noise voltage-clamp circuit using a FET input amplifier⁹. The resultant current noise signal was sampled at a frequency of 500 Hz. The power spectrum of a time series of 2,048 data points was calculated on a PDP11/34 computer. The final spectra represent an average of 40 records.

With Na Ringer's on both sides, we observed a spontaneous lorentzian component in the power spectrum in 30 of 95 bladders (curve *a* in Fig. 1A). The plateau value (S_0) and the corner frequency (f_c) could be quite accurately determined in 16 bladders: $S_0 = 3.7 \pm 0.8 \times 10^{-19} \text{ A}^2 \text{ s cm}^{-2}$ and $f_c = 4.0 \pm 0.2 \text{ Hz}$. In many cases, as in curve *a* of Fig. 1B, there was also a low-frequency noise component. In most of the experiments the lorentzian component was either masked by low-frequency noise or its intensity was too small to be detectable. The lorentzian noise component could be caused by one of the following mechanisms: (1) conductance fluctuations in one of the cell membranes or in the paracellular shunt produced by 'open and close' kinetics of ion-selective channels; (2) fluctuations in the active ion-transport flux in the case of an electrogenic transport mechanism. The relaxation time, τ , of the process we measured can be calculated from f_c ; thus, $\tau = 1/(2\pi f_c)$, being in this series of experiments $41 \pm 2 \text{ ms}$. The addition of a metabolic inhibitor (3 mM KCN + 3 mM sodium iodoacetate) to the serosal solution resulted in a complete disappearance of the lorentzian component within 20 min (Fig. 1A). The application of 1 mM ouabain to the serosal solution caused a slow decrease of S_0 with a concomitant increase of f_c (Fig. 1B). From these experiments it cannot be concluded that the underlying transport mechanism is an active one because the inhibition of active transport reduces the electrochemical gradients across the mucosal and the serosal membrane². Consequently, it is possible that the metabolic inhibitors and ouabain cause an indirect decrease of S_0 by reducing the driving force for passive ionic movements. The increase of f_c after ouabain cannot be explained with the available data.

In most of the remaining bladders we investigated, large low-frequency components were recorded and a lorentzian component could not be detected with Na Ringer's on both

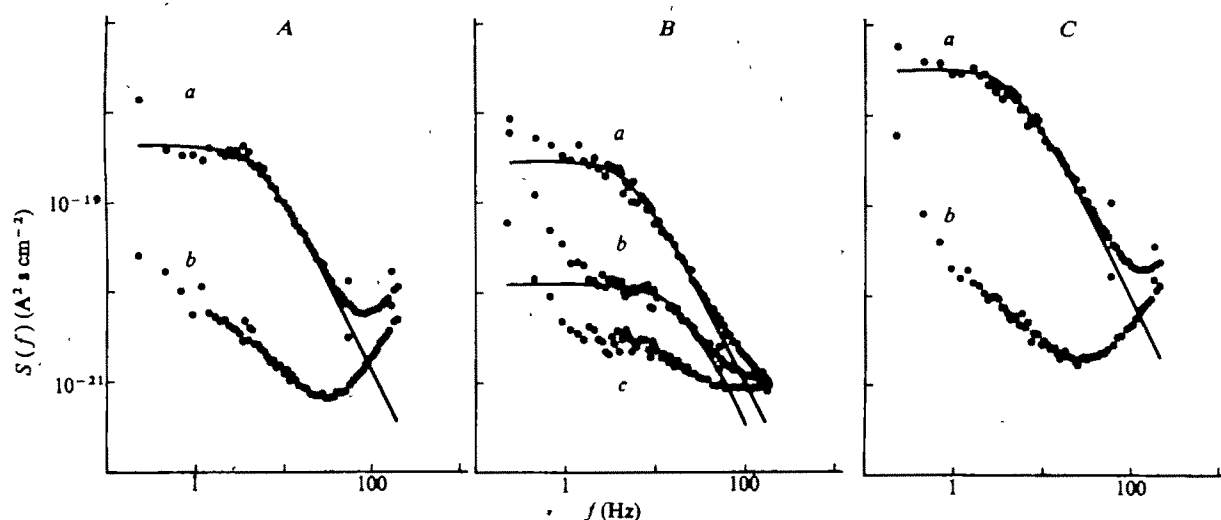


Fig. 1 Power spectra of the current fluctuations through the toad gallbladder epithelium in control conditions and after the addition of various agents. **A**, Curve *a*, spontaneous lorentzian noise spectrum recorded with Na Ringer's on both sides. The plateau value is $S_0 = 4.3 \times 10^{-19} \text{ A}^2 \text{ s cm}^{-2}$ and the corner frequency $f_c = 5.3 \text{ Hz}$. Curve *b*, fluctuations are depressed to levels comparable to the amplifier noise after the addition of 3 mM KCN + 3 mM sodium iodoacetate. Because of the capacitive reactance component in the membrane impedance, the amplifier noise increases in the high frequency range and may become larger than the excess noise of the preparation¹⁵. This explains the increase of the spectral density observed at higher frequencies. **B**, Influence of ouabain. Curve *a*, spectrum recorded in control conditions: $S_0 = 2.7 \times 10^{-19} \text{ A}^2 \text{ s cm}^{-2}$ and $f_c = 5.7 \text{ Hz}$. Curve *b*, 15 min after the addition of 1 mM ouabain to the serosal solution: $S_0 = 1.2 \times 10^{-20} \text{ A}^2 \text{ s cm}^{-2}$ and $f_c = 17.1 \text{ Hz}$. Curve *c* was recorded 35 min after the addition of ouabain. **C**, Influence of TEA. Curve *a*, lorentzian recorded with Na Ringer's on the serosal side and with Ringer's in which all Na^+ was replaced by K^+ on the mucosal side: $S_0 = 3.1 \times 10^{-18} \text{ A}^2 \text{ s cm}^{-2}$ and $f_c = 4.7 \text{ Hz}$. Curve *b*, the addition of 5 mM TEA to the mucosal solution abolished the current fluctuations.

sides. The low-frequency component could be depressed by replacing 36 mM of the serosal Na^+ by K^+ . It cannot be excluded that these fluctuations, which we shall not discuss further, are due to smooth muscle activity, and consequently can be depressed by a K^+ -induced depolarisation. A lorentzian could be shown in 71% of these preparations after replacing all Na^+ by K^+ at either the mucosal or the serosal side. This indicates that the underlying mechanism depends on the presence of potassium in the bathing solutions. We therefore investigated the dependence of S_0 and f_c on the mucosal ($[\text{K}]_m$) and the serosal ($[\text{K}]_s$) K^+ concentration. These experiments were done with bladders which showed a spontaneous lorentzian component in the power spectrum. During all Na^+/K^+ replacements f_c did not change significantly. The substitution of 36 mM of the mucosal Na^+ by K^+ led to a complete disappearance of the lorentzian component (Fig. 2). The increase of $[\text{K}]_m$ reduces the electrochemical gradient across the mucosal membrane¹¹, so that possibly the K^+ efflux to the mucosal side decreases. A further increase of $[\text{K}]_m$ enhanced the fluctuations, and the lorentzian component reappeared. At $[\text{K}]_m = 115 \text{ mM}$, S_0 was even larger than in control conditions. In these circumstances a K^+ influx through the mucosal membrane can be expected. An increase of $[\text{K}]_s$ led to an increase of S_0 over the entire range of K^+ concentrations. Even after poisoning the epithelium with a metabolic inhibitor, the lorentzian component could be observed in the spectra when a large K^+ gradient was applied.

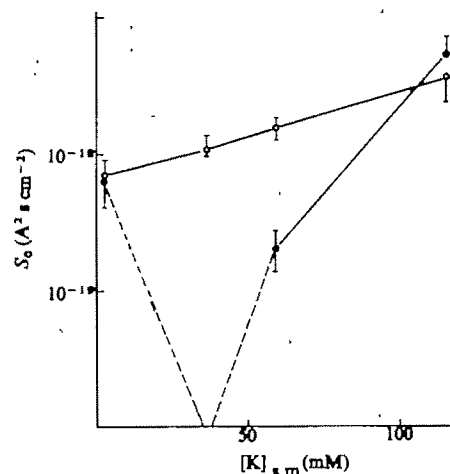
From these data it can be concluded that the relaxation process underlying the lorentzian component is related to K^+ movements through the mucosal membrane. The gradual rise of S_0 with $[\text{K}]_s$ can be understood because the increase of $[\text{K}]_s$ depolarises the intracellular potential² and possibly leads to an increase of the cellular K^+ concentration. Consequently, the increased electrochemical gradient for K^+ across the mucosal membrane results in a rise in the K^+ efflux.

More evidence for the existence of K^+ -selective channels in the mucosal membrane was found in the following experiments. It is known that tetraethylammonium (TEA) blocks the K^+ channels in nerve membrane¹² and reduces the permeability of the mucosal membrane of the gallbladder¹. The addition of 5 mM TEA to the mucosal bathing solution completely

inhibited the lorentzian noise components (Fig. 1C). This was observed for the spontaneous lorentzian current fluctuations recorded with Na Ringer's on both sides, as well as in experiments where the lorentzian was produced by a K^+ gradient across the epithelium. The inhibitory effect of TEA occurred in less than 5 min and was completely reversible. Added to the serosal side, TEA had no effect on the fluctuations. This clearly demonstrates that the fluctuating K^+ channels are located on the mucosal side.

As the paracellular shunt path of the gallbladder has a high conductance¹³, especially for K^+ (ref. 2), we investigated the possibility that the fluctuations are caused by a K^+ flux through

Fig. 2 Plateau values, S_0 , as a function of the serosal ($[\text{K}]_s$) and mucosal ($[\text{K}]_m$) K^+ concentration. Na^+ was replaced by K^+ at constant ionic strength. Closed circles represent the K^+ replacements at the mucosal side with normal Ringer's on the serosal side. The open circles are data obtained with normal Ringer's on the mucosal side and with different $[\text{K}]_s$ values. At $[\text{K}]_m = 36 \text{ mM}$, S_0 was smaller than the amplifier noise level, which is about $1.0 \times 10^{-21} \text{ A}^2 \text{ s cm}^{-2}$. As the exact position of this point is unknown, it is indicated with dashed lines, s.e.m. values are indicated.



the shunt. The cationic permeability of the paracellular pathway can be decreased with the addition of 20 mM 2,4,6-triaminopyrimidine (TAP) to both sides of the epithelium¹⁴. With Na Ringer's on both sides, the transepithelial resistance increased in this series of experiments from 128 ± 20 to $355 \pm 38 \Omega \text{ cm}^2$ ($P < 0.0005$, $n = 8$). When all Na^+ was replaced on both sides by K^+ , an increase from 169 ± 46 to $251 \pm 67 \Omega \text{ cm}^2$ ($P < 0.01$, $n = 6$) was found. We therefore assume that TAP reduces the K^+ permeability of the paracellular shunt, as was shown for other cations¹⁴. The plateau values of the lorentzians recorded either with Na Ringer's on both sides, or in the presence of a K^+ gradient across the epithelium, did not change significantly with the addition of 20 mM TAP to both bathing solutions. Therefore, as TAP reduces the paracellular K^+ fluxes and does not change the lorentzian current fluctuations, it seems unlikely that the K^+ -selective structures are located in the paracellular pathway.

From our data, we conclude that K^+ -selective channels, which open and close randomly, exist in the mucosal membrane of the toad gallbladder. No rectification characteristics could be shown, as both an inward- and an outward-directed K^+ gradient across the mucosal membrane caused an increase of the lorentzian current fluctuations. TEA, as in other tissues, acts as a reversible blocker for the K^+ channels. Further investigations, which allow us to determine the probabilities for the open and closed state, can give an insight into the mechanism which controls the K^+ permeability.

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Absence of measles proviral DNA in systemic lupus erythematosus

MEASLES VIRUS usually causes an acute self-limiting infection in humans and a productive cytolytic infection in susceptible tissue culture cells. However, measles-like virus has been isolated from the brains of patients with subacute sclerosing panencephalitis (SSPE), a chronic progressive neurological disorder of children¹. Also, persistent measles infection has been implicated in the pathogenesis of several chronic diseases of unknown aetiology, including multiple sclerosis² and systemic lupus erythematosus (SLE)³. Manipulation of the conditions of infection can result in persistently infected tissue culture cells, which have been proposed as a model system for studying the molecular mechanisms responsible for cytolytic virus persistence in man^{4–6}. There is experimental evidence for at least four mechanisms leading to the induction of persistent measles infections: the production of defective virus particles which interfere with the replication of complete virus⁷, the generation of mutant virus strains with the capacity for non-cytolytic replication⁸, and infection of certain cell types (for example, neurones) restricted in their capacity to support all steps in virus

replication⁹. Zhdanov *et al.*^{10–13} have proposed that measles virus persists in cells by a mechanism similar to that shown for retroviruses, that is, the synthesis of a DNA copy of the viral genome and the insertion of the proviral DNA into the host cell chromosome. This would be a novel mode of replication, as the replication of measles virus in cytolytic infections involves a double-stranded RNA intermediate. Using tritium-labelled viral RNA probes in molecular hybridisation assays, Zhdanov *et al.* have demonstrated the presence of measles virus-related DNA in chick embryo cells¹¹ and human lymphoid cells¹³ persistently infected with measles virus. Further, they detected measles virus-related DNA in leukocytes, lymph nodes, kidney and urine cell sediment from five SLE patients¹². Negative results were obtained using cells from appropriate control tissues. The obligative reverse transcriptase was found in the persistently infected cell lines and SLE tissues, and concomitant retrovirus infection was proposed as a prerequisite for persistent measles infection. Thus, these studies not only provide insights into the molecular mechanisms of measles virus persistence but suggest aetiological relationships for a fatal disease of unknown cause. However, we report here that, using a more sensitive nucleic acid hybridisation assay, we were unable to confirm the presence of measles virus-related DNA in either persistently infected cells or cells from patients with SLE.

We used a radioisotopically labelled complementary DNA probe (MV cDNA) generated from an *in vitro* reverse transcriptase reaction, using measles virus-specific mRNA as template and reverse transcriptase from avian myeloblastosis virus. Reaction conditions were selected to optimise the extent of transcription of the viral mRNA^{14,15}. The measles virus-specific mRNA (MV mRNA) used as template in this reaction was obtained by infecting CV-1 cells at a high multiplicity of infection and then treating the cells with actinomycin D and cycloheximide (for details see legend to Fig. 1). High concentrations of actinomycin D ($25 \mu\text{g ml}^{-1}$) are necessary for complete suppression of host cell RNA synthesis in infected cells¹⁶. Cycloheximide decreases virion RNA synthesis but increases the yield of viral mRNA¹⁷. Infected cells were ruptured and polyribosomes isolated by differential centrifugation. RNA was extracted from polyribosomes by caesium chloride centrifugation and poly-A-rich RNA purified by affinity chromatography. MV mRNA had a sedimentation coefficient of $\sim 20\text{S}$ in sucrose-SDS gradients and its separation into discrete species by formamide-polyacrylamide gel electrophoresis is reported elsewhere¹⁸.

MV ³H-cDNA was purified as described in the legend to Fig. 1. The cDNA was 93% single stranded, as determined by sensitivity to S_1 nuclease, and had a sedimentation coefficient of $\sim 7\text{S}$ in alkaline sucrose gradients¹⁹. Hybridisation of ³²P-labelled MV mRNA to excess MV ³H-cDNA (ratio 1:10) protected 90% of the labelled RNA from hydrolysis with RNase¹⁸. Further evidence of nearly complete transcription of the RNA template was found in the kinetics of annealing of the cDNA to the MV mRNA template (Fig. 1). The half-saturation value (C_{t1}) of the MV probe to its template was compared with the C_{t1} of polio cDNA annealed to polio virion RNA using the relationship described by Birnstiel *et al.*²⁰. A nearly complete DNA copy of polio RNA (98% of polio genome) was synthesised using the method of Kacian and Myers¹⁷. The kinetic complexity of MV mRNA was 2.3-fold greater than that of polio RNA. As the molecular weight of polio RNA is estimated to be $\sim 2.6 \times 10^6$ (ref. 21), the calculated complexity of MV mRNA of $\sim 6 \times 10^6$ is consistent with data obtained from sedimentation analysis of virion RNA²². The thermal stability of the measles virus mRNA-cDNA hybrid was analysed by hydroxyapatite chromatography; the T_m value was 87.5°C (temperature at which 50% of hybrid eluted from the column), indicating a high degree of base-pair matching²⁵.

The specificity of the viral cDNA probe was demonstrated by a series of hybridisation experiments shown in Table 1. There was minimal hybridisation to relevant synthetic homopolymers (rA, rG) and unrelated viral and bacterial RNA, and a small

degree of hybridisation to monkey kidney cell nucleic acid (<6%). It is likely that this results from *in vitro* transcription of normal cell mRNA contaminating the MV mRNA preparation used as template. If there is non-preferential transcription of viral and cell mRNA, the percentage of the cDNA hybridising to normal cell DNA represents the fraction of the template which is cell mRNA. This fraction has varied over ~3–20% using probes generated from different MV mRNA preparations. 87% of the probe hybridised to RNA from infected cells but there was only minimal hybridisation to DNA from either lytically or persistently infected cells. A higher degree of hybridisation to RNA from lytically infected cells probably did not occur because of the presence of unlabelled negative-strand (virion) RNA which competes with the probe for positive-strand viral mRNA in the hybridisation reaction. Removal of negative-strand viral RNA from the RNA preparation by oligo-dT cellulose chromatography increases the hybridisation of the probe to 94%.

We attempted to hybridise the MV cDNA probe to DNA from various organs (spleen, kidney, placenta) of 15 patients with SLE, and DNA from six normal individuals (that is, the organ demonstrated no histopathology). With the exception of four patients from whom placenta was obtained, all patients had aggressive disease and the majority of specimens were obtained at necropsy. The conditions of the annealing reactions were as described in the legend to Fig. 1. Annealing mixtures contained 210 µg of cell DNA and 400 pg of ³H-cDNA, and were incubated to a C_0t value of 5,000. We found that <5.2% of the probe hybridised to the DNA samples from SLE patients compared with 1.0–5.2% hybridisation to DNA from the same organs of normal controls. Similarly, there was no difference in cDNA–cell RNA hybridisation experiments using RNA from seven SLE patients and four normal controls in the same reaction conditions as those used in the DNA–DNA hybridisation reaction. 1.0–2.5% of the probe hybridised to RNA from SLE samples compared with 2.5–3.5% hybridisation to RNA from normal controls.

Therefore, the data described here do not support the findings of Zhdanov *et al.*^{10–13}. We were unable to find evidence of measles virus-related DNA sequences in persistently infected cell lines despite the use of a nucleic acid probe with 2 logs higher specific activity than that used in their studies. The persistently infected cell lines studied by Zhdanov *et al.* showed evidence of concomitant infection with a retrovirus, as determined by the presence of 70S RNA and reverse transcriptase. We could not detect evidence of retrovirus infection in our cell lines by assays

for viral reverse transcriptase²⁸, despite previous reports that some clones of HEP-2 cells contained type 'B' retrovirus²⁹. Differences in the expression of this retrovirus-specific enzyme by the cell line could determine whether other viral RNA molecules are transcribed into a DNA form and integrated.

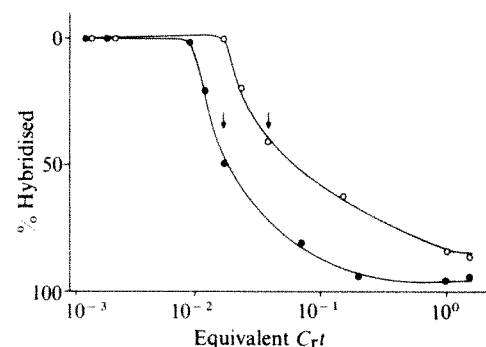


Fig. 1 Hybridisation of viral RNAs to ³H-cDNA probes. MV mRNA (○) and polio virion RNA (●) were hybridised to their respective cDNA. CV-1 cells grown in monolayer in minimum essential media with 5% fetal calf serum (MEM-FCS) were infected with the Edmonston strain of measles virus at an MOI (multiplicity of infection) of 10, for 2 h at 37 °C. Four days after infection, media were replaced with fresh MEM containing 25 µg ml⁻¹ actinomycin D (Sigma). Two hours later cycloheximide (Sigma) was added to a final concentration of 75 µg ml⁻¹. Six hours after the addition of cycloheximide, the cells were removed from the flask with trypsin and centrifuged for 10 min at 200g. They were resuspended in a solution containing 10 mM Tris-HCl, pH 7.5, 10 mM NaCl, 1 mM MgCl₂ and 5% sucrose (TNM), and kept at 4 °C for 15 min. The cells were broken by 5–20 strokes of a tight glass Dounce homogeniser and cell disruption monitored by phase contrast microscopy. Nuclei were centrifuged for 10 min at 600g and the nuclear pellet washed once more with the hypotonic buffer. The post-nuclear pellet supernatants were pooled and layered on a discontinuous gradient containing 13.4 ml of 1 M sucrose (in TNM with 100 µg ml⁻¹ polyvinyl sulphate) over 4 ml of 2.5 M sucrose in an SW27 rotor and centrifuged at 27,000 r.p.m. for 3 h at 4 °C. The opalescent polyribosomal band at the interface of the two sucrose layers was removed by puncturing the side of the tube. The polyribosomal sample was then made to 0.5% SDS and 1 M mercaptoethanol and RNA extracted by the method of Glisin *et al.*²⁴. CsCl was added at 1 g per ml of sample, and the mixture shaken for 15 min, then layered on 1.5 ml of 5.7 M CsCl (in 0.1 mM EDTA) and centrifuged at 35,000 r.p.m. for 12 h in an SW50.1 rotor at 20 °C. The RNA pellet was resuspended in 0.01 M Tris-HCl, pH 7.5, 0.5 M NaCl and poly-A-rich RNA obtained by passing the sample twice through an oligo-dT cellulose column¹⁸. The eluate from the column was pooled, made to 0.4 M NaCl, precipitated with two volumes of ethanol, and the RNA used as template in a reverse transcriptase reaction. The total reaction (0.1 ml volume) contained 50 mM Tris-HCl, pH 8.3, 50 mM KCl, 8 mM MgCl₂, 0.5 mM dithiothreitol, 1 mM each of dATP, dTTP, dGTP and ³H-dCTP (29.3 Ci mmol⁻¹, New England Nuclear), 100 µg ml⁻¹ actinomycin D, 5 µg MV mRNA, 1 µg dT₁₀ and 5 units avian myeloblastosis reverse transcriptase. For poliovirus RNA templated reactions, 35S polio RNA was used¹⁵. The reaction was incubated for 1 h at 37 °C and terminated by the addition of 0.1 M EDTA. RNA was hydrolysed and ³H-cDNA purified by methods reported previously²⁵. The specific activity of the ³H-cDNA was calculated to be ~10⁷ c.p.m. per µg DNA. Hybridisation reactions were carried out in 20–40-µl volumes in glass capillaries. The reaction mixture contained 50 mM Tris-HCl, pH 7.0, 0.5 M NaCl, 1 mM EDTA, 0.1% sodium dodecyl sulphate (SDS), 0.2 µg of viral RNA (or total *E. coli* RNA) and 4,000 c.p.m. of ³H-cDNA probe. The polio cDNA was sheared to a size comparable with that of the MVm cDNA because of the effect of single-strand length on kinetic complexity³¹. Samples were heated to 100 °C for 2 min and incubated at 65 °C. Equivalent C_0t (product of RNA concentration, time of incubation and factor for cation concentration) was determined by the method of Britten and Kohne²⁶. S₁ nuclease analysis of the hybridisation reaction was by the method of Vogt²⁷.

Table 1 Hybridisation of MV ³H-cDNA to various nucleic acids

RNA	% Hybridised
Poly rA	<1%
Poly rG	<1%
Avian myeloblastosis virus	<1%
Newcastle disease virus	<1%
<i>Escherichia coli</i>	<1%
CV-1 cells	3.6%
CV-1 cells, MV-infected	87.0%
DNA	
<i>E. coli</i>	<1%
CV-1 cells	5.7%
CV-1 cells, MV-infected	4.6%
Hamster embryo cells, MV-infected	4.0%
HEP-2 cells, MV-infected	5.2%

The hamster embryo cell line⁶ and HEP-2 cell line³⁰ were persistently infected with MV. Both cell lines produced 10²–10³ plaque-forming units per ml per 24 h and >90% of cells haemadsorbed rhesus-monkey erythrocytes. Hybridisations were carried out as described in the legend to Fig. 1, and reactions with viral RNA were taken to a C_0t = 1 and those with cell RNA and DNA to a C_0t = 5,000 (ref. 26). DNA was extracted by a method described previously²⁵ and RNA extracted by the method of Glisin²⁴.

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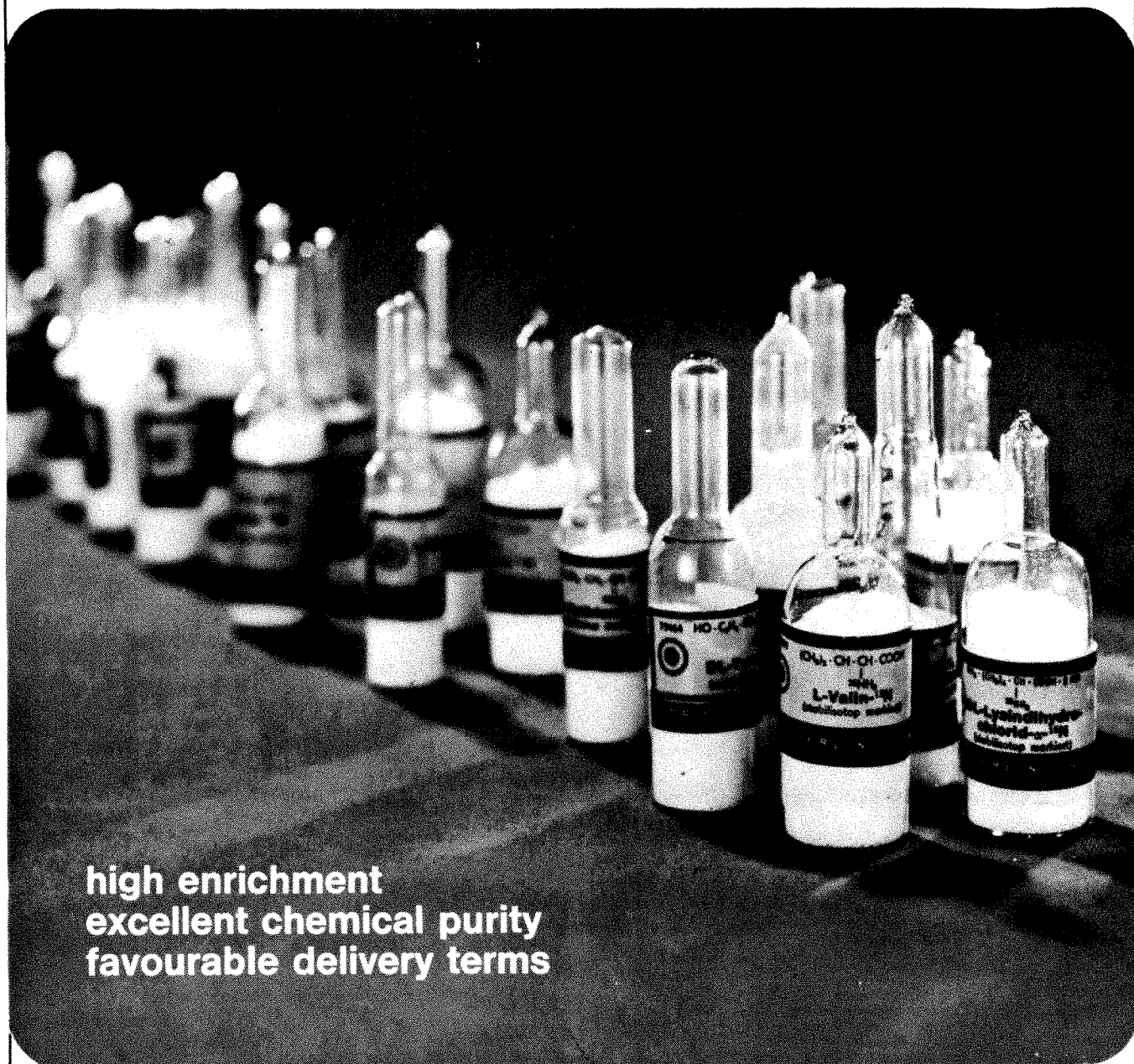
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Also, we were unable to confirm the presence of measles virus DNA or RNA nucleotide sequences in cells from patients with SLE. Alekberova *et al.*¹¹ detected 2–5 measles virus gene equivalents per cell, well within the limits of sensitivity of our assay. In reconstruction experiments, we could unequivocally detect 1 viral gene equivalent per cell. One could construct an hypothesis for a viral cause of SLE in which few cells were infected and infected cells had a low level of viral gene expression. In this situation, viral information would escape detection by the hybridisation assays carried out in the present study. However, our findings, plus evidence of the non-viral nature of the tubulo-reticular structures in SLE and the nonspecificity of increased antiviral antibody titres in this disease³, indicate that it is unlikely that measles virus has a role in the pathogenesis of SLE. Collaborative studies which are now in progress through the USA/USSR Scientific Exchange programmes should clarify the discrepancy between the results reported here and those of Soviet investigators. Further, this study has shown that viral cDNA probes can be used to examine molecular aspects of measles virus replication and persistence, and may be useful in clarifying the role of measles virus in other human diseases, particularly multiple sclerosis.

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Expression of endogenous C-type viral antigen on normal mouse haemopoietic stem cells

PARAMETERS such as size, density and differences in cell-cycle kinetics have been used to distinguish murine pluripotent haemopoietic stem cells (colony-forming units in the spleen, CFU-S) and committed haemopoietic progenitor cells¹. Efforts to use surface membrane markers^{2–4} have been based on two different, mutually exclusive concepts that regard pluripotent stem cells either as 'null-cells' or 'multiple-marked' cells. The first concept suggests that the markers are sequentially expressed during differentiation and maturation of a cell. Pluripotent stem cells, being the most undifferentiated haemopoietic cells, would be expected to have no markers or at least very few. According to the 'multiple-marked' stem cell hypothesis, however, differentiation would be accompanied by a specific loss of markers⁵. CFU-S can be detected by the development of spleen colonies after transplantation of normal syngeneic bone marrow cells (BMC) into irradiated recipients⁶. Committed granulocyte-macrophage colony-forming cells (GM-CFC) and early or late erythroid (BFU-E or CFU-E) progenitor cells can proliferate clonally in semisolid medium *in vitro* if stimulated by specific growth factors, namely granulocyte-macrophage colony-stimulating factor (GM-CSF) or erythropoietin^{7–9}. Pretreatment of normal marrow cells with a rabbit antiserum against mouse brain tissue has been reported to suppress spleen colony formation *in vitro* without influencing the growth of granulocyte-macrophage colonies *in vitro*¹⁰. We now report that antigens coded by endogenous C-type virus are expressed on pluripotent stem cells but were not detected on committed progenitor cells.

Our approach was suggested by recent experiments where an antiserum directed against endogenous xenotropic C-type virus suppressed the humoral immune response in all mouse strains tested^{11,12}. As immunocompetent cells are ultimately derived from pluripotent stem cells of the lymphoid and myeloid system¹³, it is conceivable that viral antigens are also expressed on cells of the haemopoietic system where they may have a physiological role, as suggested for cells participating in the immune response.

Fig. 1 Effect of preabsorption with virus on capacity of antiserum to suppress colony formation by haemopoietic stem cells. Marrow cells were incubated with medium or serum as described in the legend to Table 1. The final dilution of the serum was 1:80. 400 µg of density-gradient-purified virus was pelleted by centrifugation, incubated with 50 µl of serum diluted 1:10 for 60 min in ice and removed again by centrifugation. *a*, Medium control; *b*, xenotropic BALB/c virus absorbed antiserum. LPS-induced xenotropic BALB/c virus used for absorption was grown in mink cells (CCL-64); *c*, Friend leukaemia virus-absorbed antiserum; *d*, tobacco mosaic virus-absorbed antiserum; *e*, non-absorbed antiserum.

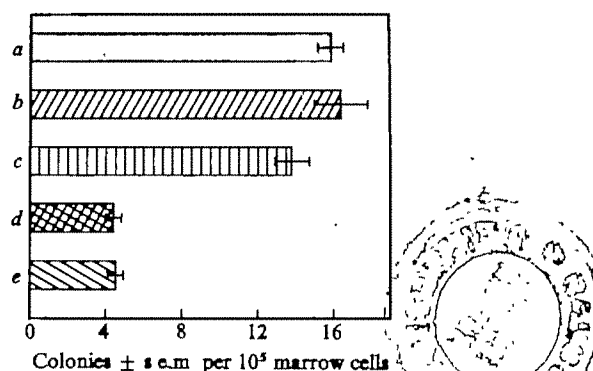


Table 1 Effect of pretreatment of marrow cells with anti-xenotropic virus serum on the number of CFU-S and GM-CFC

Serum (final dilution)	CFU-S		GM-CFC	
	Colonies ±s.e.m. per 10 ⁵ BMC	% inhibition of CFU-S	per 10 ⁵ BMC no C'	+C'
Control serum				
1:5	14.3±1.2	7.6	146	234
1:20	ND	—	112	200
1:80	ND	—	124	215
1:320	ND	—	124	186
Antiserum				
1:5	0.4±0.2	97.6	224	200
1:20	0.2±0.1	98.6	164	212
1:80	0.2±0.1	98.7	96	215
1:320	6.9±0.7	55.1	164	192
No serum	15.5±1.3	—	86	114

Marrow cells were flushed from the femurs of 12-week-old male SPF C57BL/6 mice with 1 ml RPMI 1640 medium (Microbiological Associates), supplemented with antibiotics and 0.02 M HEPES. Influence of antiserum on CFU-S: 2×10^6 viable nucleated cells were incubated at different dilutions of serum without complement (C') in a final volume of 0.4 ml at 0 °C. 30 min later the cells were diluted 1:25 with supplemented RPMI 1640 and washed. The cells were then injected intravenously into syngeneic mice pre-irradiated with 800 rad (10^5 viable nucleated cells per mouse, 15 mice per group). The spleens of the mice were removed 9 d later, fixed for 10 min in Boulin's fixative and the number of macroscopic colonies counted⁶. Influence of antiserum on GM-CFC: 3.2×10^5 viable nucleated cells were incubated with different dilutions of serum with or without complement in a final volume of 0.16 ml for 45 min at 37 °C and 7.5% CO₂. After three washings in the cold (5 min, 200g), aliquots of 1×10^5 cells were cultured in Petri dishes containing 1 ml of Dulbecco's modified Eagle's medium (Gibco) supplemented with antibiotics, and an amino acid-vitamin cocktail¹⁶ 15% horse serum (Microbiological Associates), 0.8% methylcellulose¹⁷ (Dow) and 5% mouse-lung-conditioned medium¹⁸ (representing a plateau concentration of GM-CSF) at 37 °C and 10% CO₂ in a humidified incubator. Colonies containing more than 50 cells were counted 7 d later. Each number represents the mean value of duplicate cultures. ND, not determined.

The antiserum used was from the same pool previously reported to be immunosuppressive^{11,12} and was prepared against BALB/c xenotropic endogenous C-type virus originally induced from spleen cell cultures by treatment with bacterial lipopolysaccharide (LPS)¹⁹ and collected in stably infected rabbit cornea (SIRC) cells.

To examine the effect of the antiserum on mouse CFU-S, normal marrow cells were incubated either with antiserum or control serum *in vitro*, washed and then injected into irradiated recipients. No exogenous complement was added, as it was assumed to be present in the recipients. A typical experiment (Table 1) showed that the development of spleen colonies was suppressed by pretreatment of the donor cells with antiserum. Suppression was not always complete, but with high concentrations of antiserum it was never less than 75%. In other experiments, marrow cells were incubated with antiserum and complement. After washing, cells were assayed for GM-CFC (Table 1). The antiserum did not inhibit colony development whether or not complement had been added.

In other experiments BFU-E and CFU-E frequencies were determined in marrow cells preincubated in identical conditions with combinations of antiserum, control serum and complement. Again, no influence could be detected (L. Guilbert and F.G.S., unpublished data). However, some experiments (data not shown) indicate that preincubation of spleen cells with antiserum and complement can efficiently suppress the frequency of B-lymphocyte colony-forming cells (BL-CFC). The failure of antiserum plus complement to reduce the number of colonies derived from GM-CFC or erythroid precursor cells does not definitely prove that these cells lack the corresponding antigen. The failure could also reflect low antigen density and consequent low antibody concentration on the cell-surface

insufficient to initiate complement activation. To clarify this, marrow cells were incubated with antiserum in the cold and subsequently with a goat anti-rabbit immunoglobulin (GARIG) and complement in the warm. The ability of the anti-immunoglobulin to bind complement, the lytic activity of the particular complement batch used and the efficiency of the procedure to detect low concentrations of cell-surface-bound antibody unable by itself to activate complement were tested in parallel in another system. Mouse thymus cells preincubated with a non-lytic dilution of a rabbit anti-mouse thymus cell serum were readily killed by a second incubation with GARIG plus complement. However, GM-CFC were not reduced by this procedure (Table 2). These data strongly suggest that GM-CFC do not bind significant amounts of antiviral antibodies, although

Table 2 Failure to detect endogenous viral antigen on GM-CFC by indirect cytotoxicity

Treatment of cells pre-sensitised with antiserum		Results	
GARIG (final dilution)	C'	BMC+anti-xenotropic virus serum (GM-CFC per 10 ⁵ BMC)	Thymus cells + antithymus cell serum (% cytotoxicity)
—	—	101	4
—	+	81	15
1:80	—	78	5
1:80	+	92	96

Indirect cytotoxicity for GM-CFC: marrow cells were prepared and handled during the first step as described in the legend to Table 1, but incubated with a 1:20 dilution of anti-xenotropic virus serum for 30 min at 0 °C. The cells were then washed twice (5 min, 200g) in the cold and incubated for 45 min at 37 °C and 7.5% CO₂ in air with GARIG (Behringwerke) and complement. After two further washings with the medium, the cells were assayed for GM-CFC as described in the legend to Table 1. Each number is the mean value of duplicate cultures. Indirect cytotoxicity test for thymus cells: C57BL/6 thymus cells were prepared by standard techniques in RPMI 1640 supplemented with 2% FCS. Aliquots of 5×10^5 cells were incubated with rabbit anti-mouse thymus cell serum in a volume of 0.05 ml and a final dilution of 1:2,560 for 30 min at 0 °C in microtitre plates. 30 min later 0.1 ml of medium was added and the cells washed twice in the cold. The cells were resuspended in 0.05 ml of medium containing GARIG and complement. After a second incubation for 45 min at 37 °C, the percentage of killed cells was determined by Trypan blue dye exclusion. A minimum of 200 cells were counted per sample.

an extreme resistance to complement-mediated cytolysis cannot definitely be excluded. Support for the view that the antiserum does not react with GM-CFC was obtained when the IgG fraction of the antiserum was added directly to the culture dishes. This procedure did not influence GM-CFC but it effectively inhibited the growth of BL-CFC (data not shown).

To prove that the inhibitory effect of the antiserum on spleen colony formation was due to its antiviral specificity, absorption experiments were carried out with equal amounts of different viruses. To exclude co-absorption of possible reactivities against rabbit cell-membrane antigens, xenotropic C-type virus used for absorption was grown in mink rather than rabbit cells, and was found to neutralise the activity of the antiserum completely. Preabsorption with Friend virus was almost as effective. This was expected, as Friend virus also neutralised the immunosuppressive activity of this serum¹². Cross-reactivity between Friend and BALB/c xenotropic virus is consistent with the claim that the Friend virus genome contains sequences derived from endogenous xenotropic virus¹⁴. On the other hand, tobacco mosaic virus, an unrelated virus, had no ability to reduce anti-stem-cell activity of the serum (Fig. 1).

The data presented here show that an antiserum against endogenous C-type virus suppresses the development of spleen colonies generated by pluripotent haemopoietic stem cells. On the other hand, the antiserum displayed no detectable effect on GM-CFC and erythroid precursor cells. The most likely explanation for the observed effect is that stem cells express viral antigen and are killed *in vivo* by a mechanism involving the Fc portion of bound antibodies and possibly complement. It is tempting to speculate that endogenous viral antigens are physiologically required for the differentiation of haemopoietic cells in a similar way to that suggested for cells differentiating during an immune response^{11,12,15}. It is interesting that (Fab')₂ fragments of the antibody used have been found to suppress an *in vitro* immune response demonstrating complement independence of the phenomenon (C.M. and G. Schumann, unpublished results). This serum may be useful for understanding haemopoiesis.

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Certain rheumatoid factors react with nucleosomes

NATURAL antibodies against antigenic sites on the Fc region of human and animal IgG are referred to collectively as rheumatoid factors (RF) and they represent one of the immunological hallmarks of rheumatoid arthritis (RA). Thus, the synovial fluid and tissue of RA patients contain large immune complexes which at least in part consist of RF¹⁻⁴, and RF are present in serum in the majority of patients with definite or classical RA. Recent studies have demonstrated that purified and pepsin-digested polyclonal RF of IgG class from an RA serum (Han), in addition to reacting specifically with IgG, also bind to an antigen associated with cell nuclei from many species⁵. Furthermore, essentially 100% of the anti-nuclear antibodies (ANA) of two sera (Han, Jos) was absorbed by insolubilised human IgG; the

antibodies which could be eluted from this immunosorbant bound to cell nuclei, and this binding was specifically inhibited by Fc fragments of human IgG^{5,6}. We show here that RF from serum Jos bind to nucleosomes, the repeating histone-DNA subunit of chromatin.

Mono-, oligo- and polynucleosomes were prepared by nuclease digestion of chicken erythrocyte nuclei (see legend to Fig. 1). Chicken red cells were chosen because contamination with immunoglobulins (Ig) could be avoided by washing. Nucleosomes in the nuclear digest were fractionated into two major pools by gel filtration on Sepharose 4B (Fig. 1). Pool 1 and the 150 mM NaCl-insoluble fraction of pool 2 contained all the major histone classes (Fig. 1b, d) and essentially no non-histone proteins (Fig. 1e, f). DNA from pool 1 formed a series of bands consistent with the presence of multiples of the 200 base pair monomer DNA (Fig. 1g). The size of the DNA from pool 2 corresponded roughly to nucleosome monomers and dimers (Fig. 1i). Sepharose 4B gel filtration in 0.6 M NaCl stripped the chromatin of H1 and H5, but H2a, H2b, H3 and H4 were still present (Fig. 1c).

The nucleosomes of pools 1 and 2 were tested for their ability to absorb RF specifically. Serum Jos, which contains RF with high avidity for cell nuclei⁶, and serum Ter whose RF lack ANA activity, were used for this experiment (Fig. 2). Both pool 1 (oligo- and polynucleosomes) and pool 2 (mono- and dinucleosomes) absorbed RF from serum Jos, but not from serum Ter (Fig. 2). At saturation about 85% of RF Jos was removed; this required 0.6 µg of DNA from pool 1 and 150 µg of DNA from pool 2 (Fig. 2).

In this experiment it was possible that serum IgG bound to nucleosomes and that RF reacted with bound IgG rather than with the nucleosomes themselves. IgM from serum Jos was therefore prepared (see legend to Fig. 2). A solid phase radioimmunoassay using immunospecifically purified ¹²⁵I-labelled rabbit anti-human H(γ) chain antibodies showed that the IgM preparation contained only 0.6% IgG. Nevertheless, the two nucleosome pools absorbed the same proportion (~85% at saturation) of RF from IgM as from diluted serum (Fig. 2). As the amount of IgM used was 11.5 µg, only 69 ng (0.6%) of IgG was present. From earlier studies it is known that about 25% of IgM in serum Jos is RF⁶. This means that 2,400 ng (2.6 × 10⁻¹² mol) of IgM-RF would have to be absorbed by 69 ng (0.46 × 10⁻¹² mol) of IgG, assuming that all IgG were bound to nucleosomes and thereby rendered insoluble in 150 mM NaCl. Both these events are very unlikely, and it is therefore concluded that RF Jos do indeed bind directly to nucleosomes. Further tests supported this conclusion, as nucleosomes (pool 2) absorbed 82% of RF Jos isolated immunospecifically by elution from human IgG insolubilised on beaded Sepharose. Thus, the same proportion of RF was removed from serum, the IgM and the isolated RF fraction.

To determine whether the antigenic determinant on nucleosomes which was recognised by RF Jos required the presence of histones H1 and H5, polynucleosomes stripped of H1 and H5 were prepared (see Fig. 1b and legend to Fig. 1). This material (150 µg as DNA) which was soluble in phosphate-buffered saline (PBS), displaced 87% and 77% of RF derived from serum and IgM Jos, respectively, but none of the RF from serum Ter (Table 1). By contrast, when 1,180 µg of deproteinised double-stranded calf thymus DNA with an average size of 160 base pairs was mixed with IgM Jos (5.55 µg), only 20% of RF was displaced; this value is at the significance limit for the assay. Evidently, the determinant for crossreacting RF Jos is present on the nucleosome core which consists of DNA plus H2a, H2b, H3 and H4.

The polynucleosomes of pool 1 absorbed no anti-blood-group I activity from an isolated monoclonal IgM(κ) cold agglutinin (data not shown). This result, together with their lack of binding of RF from serum Ter, supported the conclusion that RF Jos reacted specifically with the nucleosomes.

The radioimmunoassay for RF depended on the reaction of labelled rabbit anti-human L-chain antibody with RF bound to

IgG on the tube wall. It could be argued that the labelled rabbit antibodies did not bind to RF with their active sites but with their Fc regions, which might function as an antigen for RF bound on the tube wall. However, addition of a 1,000-fold excess of nonspecific 'cold' rabbit IgG in the second step of the reaction decreased binding of ^{125}I -anti-L-chain antibody by only 5% which ruled out this objection.

The present results confirm that RF Jos recognise a nuclear antigen in addition to IgG, and establish that the majority (85%) of these polyclonal RF belong to the crossreacting population. Furthermore, the data indicate that the nuclear antigen is found on a complex consisting of DNA and the four core histones H2a, H2b, H3 and H4. This conclusion is based on the following observations. About 2,400 ng of IgM RF (2.6×10^{-12} mol) was absorbed by only 600 ng (with respect to DNA) of nucleosome pool 1 (Fig. 2); this corresponds to 6×10^{-12} mol of DNA with a size of 200 base pairs, which is the DNA content in the monomeric nucleosome¹². This nucleosome fraction contained only a trace of non-histone proteins (Fig. 1e), it still bound RF after H1 and H5 had been removed (Table 1), and RF Jos did not bind to native DNA alone⁶.

The structural basis for this remarkable crossreaction is still unclear. There has been no report showing homology between sequences of the Fc(γ) region and any of the existing histones, although some similarity has been proposed between a phylogenetically ancient H4 peptide and certain V-region sequences shared by human κ and λ Ig L-chains¹³.

However, as human L-chains do not displace RF Jos from nuclei while Fc(γ) does⁶, this homology cannot explain the

Table 1 H1 + H5 stripped polynucleosomes bear antigenic determinant for rheumatoid factors (RF) in serum Jos

Antibody	H1 + H5 stripped nucleosomes*	RF bound (c.p.m.)	RF displaced† (%)
Serum Jos 1/800	—	21,899	—
Serum Jos 1/800	+	13,366	87.5
IgM Jos 11.5 μg	—	13,931	—
IgM Jos 11.5 μg	+	9,876	77
Serum Ter 1/200	—	15,117	—
Serum Ter 1/200	+	15,411	0
Normal serum 1/200	—	3,000	—

0.15 ml (150 μg) of H1 + H5 stripped polynucleosomes in PBS, pH 7.0, was mixed with 1.1 ml antibody dilution in PBS containing 1% BSA, and incubated for 2.5 h at room temperature. The mixture was then assayed for RF activity, and the % displacement calculated as described in the legend to Fig. 2.

* Chromatin fragments subjected to gel filtration in 0.6 M NaCl (see legend to Fig. 1).

† Based on comparison of c.p.m. bound by dilutions of the RF preparation in the absence of inhibitor (see legend to Fig. 2 for RF assay).

crossreaction. It is also unlikely that the determinant is a carbohydrate, as histones are not glycosylated. Possibly the two determinants recognised by the polyclonal RF Jos do not display that close structural similarity which past experience with cross-reacting antigens would lead one to expect. This is apparently the case for the polyclonal rabbit antibodies induced by immunisation with the hapten dinitrophenol, a large proportion of which crossreacts with menadione (vitamin K3)¹⁴; in this instance, the structural similarity between the two haptens is

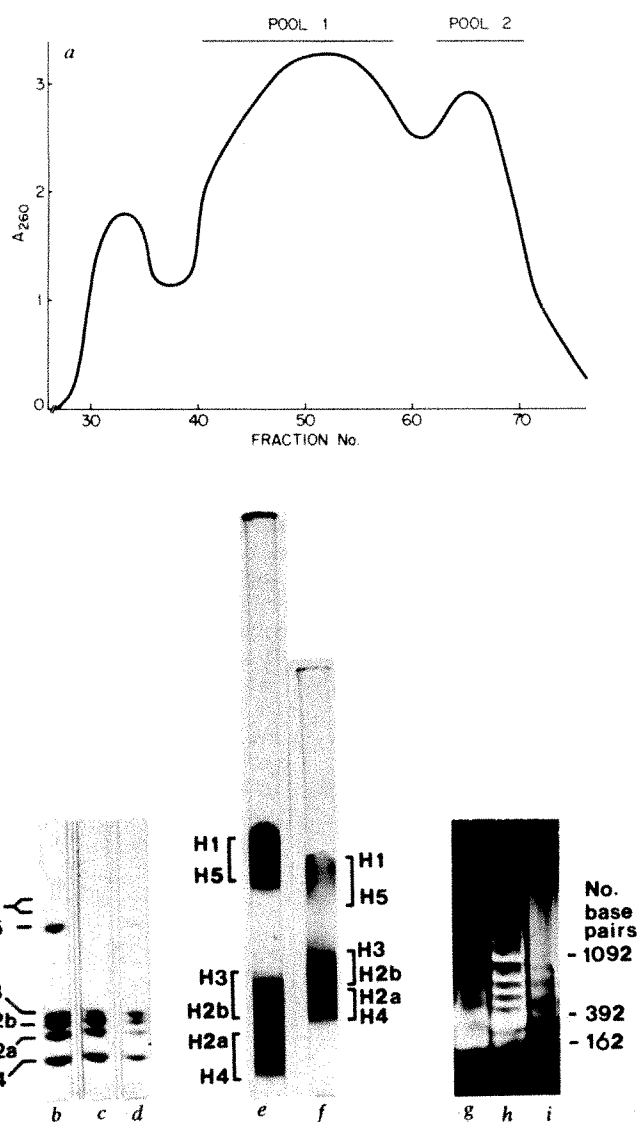


Fig. 1 Sepharose 4B gel filtration of chromatin extracted from micrococcal nuclease-digested chicken red cell nuclei. Adult chicken red cells were washed three times and nuclei prepared according to Shaw *et al.*⁷. The nuclei were resuspended in digestion buffer (0.3 M sucrose, 0.01 M Tris-HCl, 0.75 mM CaCl_2 , pH 7.2) to give $A_{260} = 70$ (read as diluted in 0.1 M NaOH) and digested for 10 min at 37°C with 20 units of micrococcal nuclease (Worthington) per 24 A_{260} units. The digestion was terminated by addition of disodium EDTA to a final concentration of 0.01 M and chilling samples in an ice bath. The suspension was centrifuged at 12,000g for 15 min. The pellet was resuspended in 0.2 mM EDTA, pH 7.0, incubated overnight at 4°C and centrifuged at 10,000g for 10 min. The extracted chromatin fragments of the supernatant were subjected to gel filtration on Sepharose 4B (7 ml supernatant containing 455 A_{260} units on a 2.5×75 cm column equilibrated with 10 mM Tris-HCl, 0.2 mM EDTA, pH 7.4). The fractions (5 ml) were pooled as indicated by the horizontal bars and concentrated by negative pressure filtration. 96% of pool 1 was insoluble in 150 mM NaCl. Of pool 2, 60% was insoluble in 150 mM NaCl, and only this fraction was used; it was redissolved in 2 mM EDTA and dialysed against 0.2 mM EDTA, pH 7.0. Another 7-ml aliquot of the supernatant was fractionated on the same Sepharose 4B column, now equilibrated with 0.6 M NaCl, 10 mM Tris-HCl, 0.2 mM EDTA, pH 7.4. The elution profile (a) was almost identical to that shown with the low ionic strength buffer; fractions corresponding to pool 1 were dialysed against PBS. DNA from the various pools was extracted with an equal volume of redistilled phenol saturated with H_2O . b, c and d show the histone composition of pool 1 (b), pool 1 from 0.6 M NaCl gel filtration (c), and 150 mM NaCl insoluble pool 2 (d). Sodium dodecyl sulphate (SDS)-polyacrylamide gel electrophoresis was carried out⁸. e, f show the purity of pool 1 (e) and 150 mM NaCl-insoluble pool 2 (f) analysed on SDS-7.5% polyacrylamide gels⁹ loaded with 60 μg (with respect to DNA) sample and stained with Coomassie blue; g, h, i show DNA fragment sizes of pool 1 (g), *HincII* digest of DNA from phage X174 (New England Biolabs) (h) and 150 mM NaCl-insoluble pool 2 (i). Electrophoresis of DNA was carried out in 1.4% agarose slab gels¹⁰ containing 40 mM Tris, 5 mM acetate, 1 mM EDTA, pH 7.9, and DNA bands were stained with ethidium bromide 1 $\mu\text{g ml}^{-1}$.

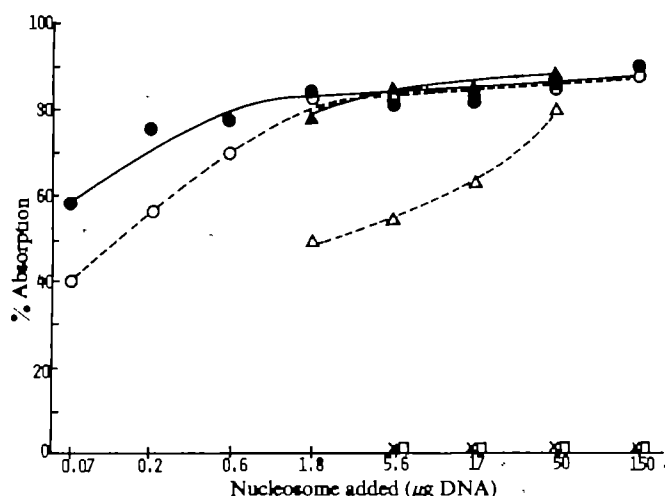


Fig. 2 Specific absorption of rheumatoid factor (RF) from serum Jos and IgM Jos by nucleosomes. IgM was prepared by gel filtration of serum on Sephadex G-200 equilibrated in 0.5 M NaCl, 0.2 M acetate, pH 4.0; the first three fractions of the breakthrough peak were pooled, and dialysed against PBS pH 7.2. Absorption of RF was effected by mixing the indicated amount of nucleosomes in 80 μ l of 0.2 mM EDTA, pH 7.0, with 1 ml of serum or IgM diluted in PBS containing 1% bovine serum albumin (PBSA). The solution turned slightly turbid immediately on addition of the nucleosomes. After incubation at room temperature for 2.5 h, the insoluble nucleosomes were sedimented by centrifugation. The supernatants were assayed for RF activity by a solid phase radioimmunoassay¹¹ using plastic tubes (Falcon 2052) precoated with rabbit IgG. The second antibody was immunoadfinity isolated ¹²⁵I-labelled rabbit anti-human L-chain antibodies. The incubation time for the first and the second antibody was extended to 18–24 h. The amount of RF absorbed by the nucleosomes was determined from standard curves obtained by making twofold dilutions of the RF-containing sample in PBSA. Absorption of 75% of RF thus corresponds to the radioactive antibody bound by a standard sample diluted fourfold. $\circ$, serum Jos diluted 1/800 + nucleosome pool 1; Δ , serum Jos 1/800 + pool 2; $\bullet$, IgM Jos (11.5 μ g) + pool 1; $\blacktriangle$, IgM Jos (11.5 μ g) + pool 2; $\square$, serum Ter 1/200 + pool 1; $\times$, serum Ter 1/200 + pool 2.

limited¹⁵ and hydrophobicity may be an essential shared component. A few homogeneous myeloma proteins and Waldenstrom macroglobulins with antigen-binding activity have also been found to possess more than one specificity^{15–16}. Thus, RF Jos and similar RF detected in other sera^{5,6} may be examples of 'polyfunctional' antibodies.

The crossreacting RF described in the present report emphasizes the general problem of extrapolating from the specificity of natural antibodies to the original antigenic stimulus which elicited their production. Moreover, the observations indicate that caution should be exercised in the interpretation of test results where RF are used as reagents, for example in detection of immune complexes.

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Hydrophobic character of amino acid residues in globular proteins

PREDICTIVE studies on the secondary structure of globular proteins aimed at locating ordered structural segments have provided little information about spatial orientations or even as to whether residues are exposed or buried. However, the physico-chemical properties¹ of residues can be used to obtain such information. In particular the hydrophobic character², is a useful parameter in these studies. The hydrophobic character as defined by the indices given by Tanford³ and Jones⁴ does not reflect hydrophobic environment within protein structures, but we introduce here a new parameter, the 'bulk hydrophobic character' obtained from an analysis of the surrounding hydrophobic environment of amino acid residues in protein crystals. This is a better index of protein hydrophobicity, showing very good correlation with the extent to which residues are buried⁵ (correlation coefficient $r = 0.9$) compared with the hydrophobic indices used previously, and it could be used to characterise tertiary structures.

Our study is based on crystallographic data on 14 protein molecules (myoglobin, ribonuclease S, cytochrome *c*, lysozyme, staphylococcal nuclease, carboxypeptidase A, subtilisin BPN', α -chymotrypsin, carp myogen, cytochrome *b*₅, apolactate dehydrogenase, trypsin inhibitor complex, concanavalin A and flavodoxin; see ref. 6 for further details on these proteins). They include representative examples of all known characteristic structural types of proteins defined by Levitt and Chothia⁷.

We demonstrated previously⁶ that a sphere of 8 Å radius has the required volume for any one residue of a protein molecule for a study of the influence of the surrounding environment. In this report, therefore, we define the 'surrounding hydrophobicity' of a residue as the sum of the hydrophobic indices assigned to the various residues that appear within an 8 Å radius volume in the protein crystal. This parameter is given by:

$$H_i = \sum_{j=1}^{20} L_{ij} h_j$$

where L_{ij} is the total number of surrounding residues of the i th type associated with the j th residue in a given protein, and h_j is the hydrophobicity index for the j th residue. All the H values that belong to the same type of residue in the 14 crystals have been grouped together, and the arithmetic average ($\bar{H}$) for each type has been calculated. These average surrounding hydrophobicities are given in Table 1, together with the corresponding individual hydrophobic indices given by Tanford³ and Jones⁴. These ($\bar{H}$) values reflect the preferred hydrophobic environments for the residues and hence their 'bulk hydrophobic character'.

Table 1 Average surrounding hydrophobicities and the hydrophobicity indices for 20 amino acid residues occurring in globular proteins

Residue	Average surrounding hydrophobicity (H) (kcal)	Hydrophobicity index (h_i) (kcal)
Ala	12.97	0.87
Asp	10.85	0.66
Cys	14.63	1.52
Glu	11.89	0.67
Phe	14.00	2.87
Gly	12.43	0.10
His	12.16	0.87
Ile	15.67	3.15
Lys	11.36	1.64
Leu	14.90	2.17
Met	14.39	1.67
Asn	11.42	0.09
Pro	11.37	2.77
Gln	11.76	0.00
Arg	11.72	0.85
Ser	11.23	0.07
Thr	11.69	0.07
Val	15.71	1.87
Trp	13.93	3.77
Tyr	13.42	2.67

In Fig. 1 the individual hydrophobic index (heavy line) is compared with the corresponding preferred surrounding hydrophobicity (thin line) standardised with respect to Gln. Both Fig. 1 and Table 1 show that the surrounding hydrophobicity is not proportional to the corresponding hydrophobic index of the residue. Taking the actual values given in Table 1, the residues can be divided into three groups: Cys, Phe, Ile, Leu, Met, and Val with (H) values greater than 14.0 kcal belong to group I. Gly, Ala, His, Trp and Tyr fall into group II with (H) values between 12.0 and 14.0 and are thus less hydrophobic than group I residues, and the remaining residues Asp, Glu, Lys, Asn, Pro, Gln, Arg, Ser and Thr with values below 12.0 kcal belong to group III. Val and Ile have the highest surrounding hydrophobicity, with Leu, Cys and Met coming next in order. Though Val is less nonpolar than residues such as Ile, Phe and Trp when defined by thermodynamic transfer measurements according to Tanford, the average surrounding hydrophobicity value suggests that this residue can easily be accommodated inside the molecule. The low value of (H) for Trp and Tyr indicate that one polar atom even in a large nonpolar side chain is sufficient to cause a large reduction in the hydrophobic environment. When considering the residues in group III, it is important to note that the surrounding hydrophobicity of Pro lies within the range observed for the polar residues. As the characteristic structure of proline does not allow it to occur in

either β strands or helices except at the N terminus, it is restricted to loops that inevitably lie on the surface thus lowering the surrounding hydrophobicity. Of the polar residues Gln, Glu and Arg have higher hydrophobicities, and the preferred environments for the charged residues Asp and Lys are associated with the lowest surrounding hydrophobicities.

Our results show that Val with an hydrophobic index almost half that of Trp, has the highest surrounding hydrophobicity. On the other hand, the surrounding hydrophobicity of Trp is very much reduced even though this residue appears at the top of the hydrophobicity index scale. The same conditions prevail for residues Tyr and Pro which, in spite of high hydrophobic indices, exhibit surrounding hydrophobicities similar to those of more polar residues. These observations suggest that apart from the nonpolarity indicated by the hydrophobic indices of amino acid residue side chains, both their structure and the presence of some polar atoms strongly influence their bulk hydrophobic character and hence their spatial position in protein molecules. Surfaces of globular proteins often contain several nonpolar residues^{1,2}, mainly group II residues. In our earlier report³ we pointed out that surface nonpolar residues occur as short distorted α -helical and extended structural domains (one to three residues long) with the backbone polar atoms having high solvent accessibility. The (H) parameter therefore characterises the hydrophobic environment preferred by each amino acid residue in a globular protein and we suggest that this specific preference for each residue is an important factor in the folding of a linear chain into a globular shape. We are now studying applications of this parameter to protein folding.

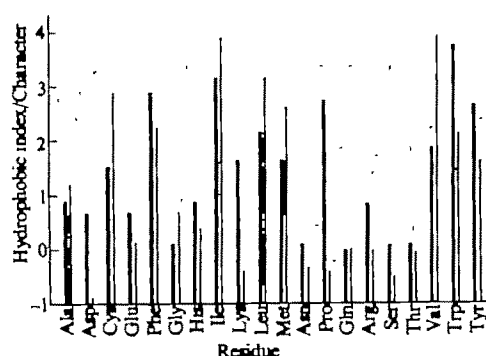
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Fig. 1 Comparison of the average bulk hydrophobicity of amino acid residues with their corresponding hydrophobic indices: thin line, average hydrophobicity; broad line, hydrophobic index.

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reviews

Fenland ecology

Peter D. Moore

Fenland: Its Ancient Past and Uncertain Future. By Sir Harry Godwin. Pp. 196. (Cambridge University Press: London and Cambridge, 1978.) £7.95.

THERE is no better living demonstration of the interaction of man with the natural environment anywhere in Britain than in the fenlands of East Anglia. Throughout prehistory and history these extensive tracts of swamp-land and bog have proved both a limitation on human mobility and settlement and, in more recent times, a valuable agricultural resource. A number of books, especially those of H. C. Darby, have considered man's conflict and symbiosis with fenland, particularly from the historical geography point of view, but this book approaches the subject from the biological angle.

In this sphere, Sir Harry Godwin is the ideal person to write such a book, for the story of the unravelling of fenland's history is essentially Godwin's autobiography. Indeed, the book has a strongly autobiographical, almost anecdotal style. It is written in an informal manner for a general readership; it avoids technical terms and jargon and does not carry references in the text, although a reading list is given at the end. This makes it easy, even exciting reading, which should prove attractive to students.

One advantage of the relaxed style is that we are provided with some rare glimpses into those chance events in history which have profoundly influenced the development of ecology in this country. Take, for example, that priceless sentence which introduces the chapter on pollen analysis, "In 1931, following a suggestion from A. G. Tansley, my wife began research in pollen analysis, a technique recently brought to notice by the Swede G. E. Erdtman . . .". It is difficult now to assess the full consequences of Tansley's chance remark.

Much of the book is concerned with the stratigraphy and palaeoecology of the fenland sediments. Rooted in the basal clays of the fens at many sites were found the preserved remains of giant bog oaks, many having dimensions far exceeding those found commonly in modern British forests; often they were over 27 metres in height.

These were inundated and killed by rising water tables as the true fens were formed. Subsequently, further woodland of a swampy nature developed over the reed bed sediments, and the sequence of species in these fen woods, leading ultimately to the establishment of pine, is of particular interest, for one finds similar developments on the other side of the North Sea and in some of the west coast submerged forests, as at Borth and Ynyslas, Dyfed.

The archaeological interest of the fenland peats is again introduced by a personal reminiscence: "I recall picking out (from the borer) what I took to be a bit of pine bark: licking it clean of peat . . . it shewed itself to be a struck flake of flint." Thus began the excavations of Shippea Hill and Peacock's Farm in fenland, now classic archaeological sites.

One of the most interesting questions in peatland ecology raised by Godwin's fen studies, is the extent to which true raised bog communities once flourished in the east of Britain. Peat cutting and agricultural developments have destroyed much of the evidence of past acidification of the fen systems leading to bog formation. At Woodwalton Fen, Godwin describes the surviving stratigraphic evidence for this process and at this site there still remain some frag-

ments of a relict, acidophilic community on the surface.

The latter part of the book concentrates on the hand of man in the modification of the fenland environment, and on the habitats and species lost as a result of exploitation. Perhaps the most important message of this book as far as mire conservation is concerned is the vulnerability of the ombrotrophic, raised mire system. The loss of this habitat in eastern England cannot be repaired; such a late stage in mire succession takes millennia to develop and the lesson of the fens should serve as a warning to those concerned with mire conservation in northern and western Britain, where similar exploitation is now occurring and where similar consequences are to be feared.

This book is essentially a compilation of scattered, largely previously published material, which is presented in a lucid, informal and readable manner. It is not so much a textbook as a personal view of a distinctive part of Britain and of the development of a field of environmental research in which the author has himself played the leading role. □

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Mathematical models in immunology

Theoretical Immunology. Edited by G. I. Bell, A. S. Perelson and G. H. Pimbley, Jr. (Marcel Dekker: New York and Basel, 1978.) Sfr. 128.

GEORGE BELL'S mass action model of the immune response made it respectable to use computer simulation in immunology. Now eight years later we have an opportunity to see how the field has progressed. This volume provides the first way of doing this conveniently, and as such can be recommended to professionals and general students. Although multi-authored, it is not a miscellaneous collection, but has a logical sequence with useful introduction and indexes. Several of the papers come from Bell's

own group of renegade theoretical physicists at Los Alamos.

As judged from this collection, mathematical models contribute more to immunology than to, say, embryology, and less than to fisheries research. Reasonable assumptions about lymphocyte triggering and regulation generate models which fit well empirical data on affinity maturation, high and low zone tolerance, and cycling antibody production. The models seem to be flexible to an extent which augurs well for the future as new phenomena are discovered, but which makes one a little uneasy at present. Thus, some models run quite satisfactorily without network suppression (Bruni, Mohler), whereas others depend on this entirely (Richter, Hoffman). Another difficulty is that in their latest version as expounded here, some of the models have grown mathematically rather

baroque.

A second group of theories deal with useful treatments of the MIF and Jerne plaque assays, the latter showing that several assumptions commonly made by experimentalists are probably wrong. Some common cook-book analysis—for example, of antibody affinity—is omitted. A third group deal with local phenomena: B cell triggering, cell recruitment (including a particularly elegant treatment of lymphocyte traffic), and antibody fit. Inman's handling of the antibody fit-frequency relationship improves on but

omits reference to my earlier work.

The weakest part comes from the experimentalists, who should have provided balanced reviews. Dual recognition is not as obscure as Burnet and Dutton make out, but does provide a problem for helper factors which Feldmann skips. The networkers have yet to take up Raff's challenge (*Nature*, 265, 205; 1977).

N. A. Mitchison

N. A. Mitchison is Professor in the Tumour Immunology Unit, Department of Zoology, University of London, UK.

Barley handbook

Barley. By D. E. Briggs. Pp. 612. (Chapman and Hall: London, 1978.) £25.

BARLEY is one of those crops, like wheat or cotton, with a fascinating biology and a complicated technology to match. Dennis Briggs makes a valiant attempt at a monographic treatment of all aspects of this, the world's fourth most important cereal crop. His book is a useful addition to the literature, although almost inevitably it suffers from unevenness.

The book begins with chapters on the morphology of the vegetative and reproductive plant. These are welcome, although it is unfortunate that the relevance and quality of some of the diagrams, often taken from rather elderly works, is doubtful; a page of illustrations of types of hood found in barley seems excessive, and better drawings, or even scanning electron micrographs, of the developing ear would have been valuable. The origin and classification of barley is discussed in a brief chapter and barley genetics are considered as a prelude to a chapter on crop improvement. To a non-specialist the treatment seems rather superficial, but a great deal of the recent literature is quoted, and this is a feature of the book as a whole.

Three chapters on barley biochemistry, grain quality and germination, and growth of the barley plant occupy about a third of the book. They are informative and give a comprehensive picture of almost every aspect of barley development and metabolism, at the price of being rather unselective and presenting biochemical pathways in somewhat exhaustive detail.

Agricultural aspects of the barley crop take up five chapters. That on agricultural practices and yield is brief and on occasions rather trivial. It is complemented by chapters on weeds, pests and diseases, and problems of

storage. Here one looks in vain for data on estimates of crop losses due to plant and insect pests, but perhaps in consolation we are given a formidable list of diseases carried by the rat. This rather unsatisfactory section also includes a chapter on production and harvesting machinery which is of doubtful value. No-one is going to go

Diatomic molecules

Diatomic Molecules: Results of Ab Initio Calculations. By R. S. Mulliken and W. C. Ermler. Pp. 197. (Academic: New York, San Francisco and London, 1978). \$19.50.

It seems churlish to greet any publication which has Robert Mulliken as an author with something less than total enthusiasm. His contributions to molecular physics have been truly monumental and his personality has inspired affection in those who know the man and his work. The choice of topic for this book again raises high expectations.

Diatomic molecules have always been wonderful model systems on which to base and test theories. Their spectroscopy is so rich and the results are so accurate and unambiguous that they have always attracted high calibre experimentalists as well as theoreticians. Nor is their importance declining: far from it, as the advent of lasers has provided tools for even more subtle experiments. The diatomic molecule has been the subject of vast numbers of experiments and its importance breaks out from the vicious circle of experiments being done for theoreticians who do calculations to explain experiments. Two-atom molecules are of wider interest, because of their key roles in interstellar molecule formation, atmospheric physics, energy transfer mechanisms and in the development of new laser systems.

The time is clearly ripe for a major

work on all aspects of the physics and chemistry of diatomic molecules. This is not that book, but any such publication might well be dedicated to Robert S. Mulliken.

The actual work under review provides a survey and analysis of computational studies of the electronic structure of ground and excited states of diatomic molecules. It is neither a student text nor a full monograph. It commences with a useful summary of theory which can largely be found in a number of other texts and which is probably too condensed for the novice and too familiar for the expert. Succeeding chapters treat one-electron molecules, two- to four-electron molecules, hydrides and finally homopolar and heteropolar species.

Electron correlation is emphasised as are charge distributions, but the other molecular properties which are the object of *ab initio* calculation are only given a cursory treatment. The bibliography contains some 1976 references but in the text an important 1974 paper is described as 'recent'.

Perhaps the combination of distinguished authors and important topics raises expectations which are bound to be disappointed. The book will be welcomed by the small band of theoreticians who work on diatomic molecules but will probably miss the potentially wider audience. It might just provoke someone to write or edit the major tome on all aspects of diatomics.

Graham Richards

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IRCS Journal of Medical Science

IRCS Med. Sci. Volume 6 No. 9

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and K. Domaniewska-Sobczak**

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obituary

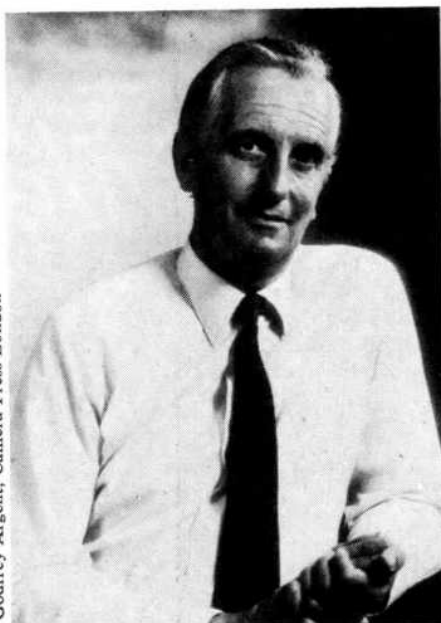
G. W. Kenner, 1922–1978

WITH the tragic death of Professor G. W. Kenner at the early age of 55 we have lost a most distinguished organic chemist, and the leading authority on peptide and protein synthesis.

George Wallace Kenner was born in Sheffield in 1922, of parents who were both chemists; his father, James Kenner, F.R.S., became Professor of Technological Chemistry in Manchester in 1928 and George spent most of his boyhood there attending the Grammar School. Although he was educated and excelled on the classical side at school, the pull of the family profession was obviously strong for he turned to chemistry when he entered University in 1939. First in Manchester and then in Cambridge he displayed prodigious talents for science, still recalled by his contemporaries. Possibly because of the breadth of his education, his early interests in chemistry covered an unusually wide span and continued to do so for the rest of his career.

His first research, in Manchester with Professor (now Lord) Todd, was on nucleotide synthesis and this, together with the synthesis of nucleotide co-enzymes, continued when the group moved to Cambridge in 1944. The structure, chemistry, and total synthesis of biologically important compounds was to remain Kenner's consuming research interest.

In 1957, as possibly the brightest of Todd's galaxy of young Cambridge stars, he came to Liverpool to the Heath Harrison Chair of Organic Chemistry, in succession to a select band of organic chemists: Sir Robert Robinson (1915–20), Sir Ian Heilbron (1920–33), and Alexander Robertson (1933–57). He found a department which, though well-known as a centre for classical natural product research, was rather run down and was very deficient in modern equipment. Sheer ability and enthusiasm gained him the support of the staff and with them he quickly built up an impressively equipped modern department. Almost immediately he started to plan the splendid Robert Robinson Laboratories



Godfrey Argent, Camera Press London

which, occupied in 1961, are still in the forefront of modern chemistry buildings and now provide a permanent monument to his life and work. Working in them nearly twenty years later, one can still sense the explosive energy at the origin of this particular part of the universe.

He showed vision and magnanimity in building up the department, in creating the right atmosphere for good research, and in the staff he appointed. Particularly imaginative was his invitation to Alan Battersby to a new Second Chair of Organic Chemistry in 1962, one of the first such appointments in the country. Their combined talents contributed greatly to organic chemistry and established the department as an international research centre which, in the twenty-one years of Kenner's leadership, expanded beyond recognition.

During this time he also played an important part in undergraduate teaching and in University affairs. While he served skilfully on many major boards and committees, he was not a committee man. He trusted more to the benign dictatorship of departmental

heads and provided a splendid example of that system at its best. He became steadily less tolerant of recent democratic trends in Universities and the associated bureaucracy; his Royal Society Research Professorship, which was the first ever held in Liverpool, brought a welcome relief from many administrative chores.

He was deeply dedicated to his work and expected much of his associates; indeed he was fond of equating academic freedom with the freedom to work still harder! Sadly, the standards he demanded of himself were often unattainable and he sometimes suffered, as perfectionists must. He always said and did exactly what he thought right, regardless of personal considerations and expediency. He was, nevertheless, a splendid colleague, charming and unfailingly courteous, lively and forceful, and there was rarely a dull moment in his company! He had a superb command of English, spoken and written, and his early classical training often erupted with flair, never more pungently than when dealing with sloppiness of thought or action, from whatever source. Even away from the laboratory George did not do things by halves, and he gained immense pleasure from sailing, hill walking, and driving, buying and selling fast motor cars and motor-bikes.

His scientific originality and judgement were widely respected and his services were constantly in demand by industrial, academic and professional organisations. He was a much valued industrial consultant and a champion of genuine collaboration between academic and industrial research, for which he greatly admired the Swiss. His extensive and important scientific work in Liverpool, especially in the fields of porphyrin and protein synthesis, culminated in the present herculean task of total synthesis of a protein closely related to the natural enzyme lysozyme, with the aim of shedding light on the mechanism of lysozyme action. His synthetic techniques and criteria of purity were those of the rigorous organic chemist since

he emphatically rejected the view that standards could be relaxed when the molecules became large and complex. The linear sequence of Liverpool lysozyme's 129 amino acids, fully protected, has been constructed and the final, though demanding, phase of the work is concerned with removal of the protecting groups. Although he will not see the conclusion of this pioneering work, he had already brought new scientific standards to the art of peptide synthesis and provided a firm foundation on which others can, and undoubtedly will, build in the future.

This work had brought him most honours and distinctions available to a British chemist. He was awarded the Meldola and Corday-Morgan Medals, gave the Tilden, Simonsen, and Pedler Lectures of the Chemical Society, and the Bakerian Lecture of the Royal Society. This last, *Towards Synthesis of Proteins* (*Proc. R. Soc. A* 1977, **353**, 441), provides a fascinating account of the strategy and achievements of the lysozyme project.

In spite of the constant demands upon him he always found time to help with the countless problems brought to him. He had an impressive memory for all who passed through the department and he followed their subsequent careers with genuine interest. Recent tragic events have shown just how many people respected and revered him, and are now left, with his wife and daughters, to mourn this splendid man.

C. W. Rees

M. V. Keldysh

MSTISLAV VSEVOLODOVICH KELDYSH, former President of the Academy of Sciences of the USSR, died on 24 June 1978.

Keldysh was born in Riga in 1911. He graduated from the Faculty of Physics and Mathematics in Moscow State University in 1931, and then went to the Zhukovskii Central Aerodynamics Institute, where he worked on mathematical aerodynamics, including the theory of unstable wing movements and flutter.

Although Keldysh was later to be acknowledged as having played a founding role in the establishment of post-war Soviet technology—computer mathematics, nuclear engineering and space research—he remained, to the outside world, relatively unknown, save as a mathematician, until 1961, when he became President of the Soviet Academy of Sciences. Although the Academy still elects its President by

secret ballot, Keldysh's appointment was largely seen abroad as being associated with expansion of the Soviet space programme.

As President of the Academy he played a major role in the expansion of Soviet science, and the development of a Union-wide network of scientific research establishments and centres. His speeches on the progress of Soviet science became a prominent feature of virtually all Party and academic occasions.

Keldysh retired from the Presidency of the Academy in 1974, shortly before the belated celebrations of its 250th anniversary. He still continued, however, to take an active part in the organisation of Soviet science, serving, for example on the Committee for Lenin and State Prizes.

For his services to Soviet science, Keldysh was honoured during his lifetime by the title of Hero of Socialist Labour (three times), Lenin and State prizes and numerous medals and orders of the Soviet Union and the countries of the socialist bloc. As a further and final tribute he was accorded a state funeral, his ashes being enshrined in the Kremlin wall.

Vera Rich

C. O. Hebb

DR CATHERINE OLDING HEBB, formerly Deputy Chief Scientific Officer, A.R.C. Institute of Animal Physiology, Babraham, Cambridge, died at Cambridge on 26 August 1978, aged 67.

Catherine Hebb was born in Nova Scotia and educated at Dalhousie University, Halifax, Nova Scotia, and at McGill University, Montreal. Here in 1937 she gained her Ph.D. and carried out her first research, on the secretory activity of the digestive glands, under Professor B. P. Babkin.

In 1938, Dr Hebb came to Edinburgh University as a Junior Beit Memorial Fellow, in the Department of Physiology, under the late Professor I. de Burgh Daly. For the next fourteen years, in collaboration with Daly and other members of his very active department, Catherine Hebb produced a series of papers on the control of the blood supply to the lungs by neural and chemical processes. During the Second World War, she and Daly undertook research in the department at Edinburgh on behalf of the Air Ministry and the Ministry of Supply. Throughout this period, until 1952, Dr Hebb was giving lectures to undergraduates

in the physiology department, including some to the students of the refugee Polish Medical Faculty in Edinburgh.

When Dr Daly was appointed first Director of the Agricultural Research Council Institute of Animal Physiology at Babraham, Cambridge, he invited Catherine Hebb to join his staff. Dr Hebb's work on the lung vasculature, together with a year's work with Professor W. Feldberg in Cambridge, during the tenure of a Senior Beit Fellowship, led her to an interest in the autonomic system and neurotransmitters, and it was in this field that her most important scientific contribution was made. She established at Babraham a first-rate neurochemical laboratory, and in 1965 became the head of the Institute's physiology department.

Chemical transmission from nerve-ending to effector organ had been familiar to physiologists for decades, but neuron-to-neuron transmission, in ganglia and in the central nervous system itself, was a newer, promising and technically difficult field when Dr Hebb began work in it. Her studies on the mechanism of the synthesis of acetylcholine, and of the distribution of the enzymes concerned in its metabolism, combined the techniques of biochemistry, histochemistry and classical pharmacology. However, among all this fine detail she never lost sight of the physiological functioning of the whole animal, and for this reason among others, she was able to write excellent review articles, putting her own discoveries and those of others in their proper context. The Physiological Society monograph on the vasculature of the lung which she wrote in collaboration with I. de Burgh Daly in 1966 is still a standard work.

Dr Hebb was notable for the sympathetic encouragement which she gave to younger workers in the field of neurochemistry, including a number from Poland, Yugoslavia, Czechoslovakia, Hungary and South American countries. She helped to bring such workers to Babraham for periods of research and study, thereby disseminating enthusiasm for the subject and also a wish to emulate her own high standards of work.

Dr Hebb had already been battling against ill-health for some ten years when she retired from Babraham in 1976. Her concern for others was typified by her request that the collection made to mark her retirement should be used to establish a loan fund to help staff, visiting scientists or students at Babraham in financial need. Contributions to this Catherine Hebb Loan Fund have flowed in from every continent—an indication of the width of the affection for Catherine Hebb and admiration for her work.

M. W. Stanier

announcements

Meetings

7 December, **Strategy for Nutrition Research**, London (Dr M. R. Turner, British Nutrition Foundation, 15 Belgrave Square, London SW1, UK).

9 January 1979, **Annual Histochemistry**, London (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford, UK).

13-15 March, **Safety of Electrical Instrumentation in Potentially Explosive Atmospheres**, Chislehurst (Mrs R. G. Keilher, Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

26-30 March, **Quantitative Microscopy (Including Image Analysis)**, Cambridge (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford, UK).

26-28 March, **Microscopy of Amorphous Solids**, Cambridge (The Administrator, Royal Microscopical Society, 37-38 St Clements, Oxford, UK).

28-30 March, **Mechanical Properties of Materials at High Rates of Strain**, Oxford (Meetings Office, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

2-6 April, **Principles of EM and Specialised Transmission EM**, Leeds (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford, UK).

9-11 April, **Microscopy of Semiconducting Materials**, Oxford (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford, UK).

24-27 April, **5th International Conference on Invertebrate Tissue Culture**, Lucerne (E. Kurstak, Département de Microbiologie et Immunologie, Faculté de Médecine, Université de Montréal, C.P. 6128, Succ. A. Montréal, P.Q. Canada H3C 3JL).

29 April-5 May, **15th EUCHEM Conference on Stereochemistry**, Burgensstock (Prof. Sir Derek Barton, FRS, Institut de Chimie des Substances Naturelles, CNRS, F-91190 Gif-sur-Yvette, France).

9 May, **3rd Annual Electron Microscopy Meeting**, Aston (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford, UK).

6-8 June, **Towards 2001—New Information Technology and Its Future Impact**, Torquay (Mike Allen, Beecham Pharmaceuticals, Coldharbour Rd, The Pinnacles, Harlow, Essex, UK).

2-6 July, **Course on Introduction to Advanced Imaging Techniques for Biologists**, Leeds (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford, UK).

8-14 July, **International Conference on Climate and History**, Norwich (Conference Secretary, Climatic Research Unit, School of Environmental Sciences, University of East Anglia, Norwich, UK).

9-13 July, **2nd International Conference on the Mechanisms of Reactions in Solution**, Kent (Dr John F. Gibson, The Chemical Society, Burlington House, London W1, UK).

9-13 July, **1st International Botanical Microscopy Meeting**, York (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford, UK).

10-12 July, **Immunocytochemistry**, York (The Administrator, Royal Microscopical Society, 37/38 St Clements, Oxford, UK).

16-18 July, **Automation in Industrial and Clinical Chemistry**, London (Beverly Humphrey, Scientific Symposia Ltd, 33/35 Bowling Green Lane, London EC1, UK).

16-19 July, **Endocrinology**, London (Conference Secretary, RPMS Du Cane Rd, London W12, UK).

Meetings, Awards, Appointments, On the Move, and Person to Person are listed on the "announcements" pages of *Nature*.

Entries for any of these sections should be sent to: *Nature*, 4 Little Essex St, London WC2, UK. (There is no charge for this service.)

The previous list of meetings was in *Nature* of 12 October, page xvii.

24-27 July, **Video and Data Recording**, Southampton (Conference Secretariat, Institution of Electronic and Radio Engineers, 99 Gower St, London WC1, UK).

30 July-3 August, **7th AIRAPT International High Pressure Conference**, Le Creusot (Andre Kusubov, University of California, Lawrence Livermore Laboratory, California 94550).

13-17 August, **Electron Microscopy Society of America: 37th Annual Meeting**, San Antonio (Dr William H. Massover, Division of Biology and Medicine, Brown University, Providence, Rhode Island 02912).

19-23 August, **3rd International Conference on the Chemistry and Uses of Molybdenum**, Michigan (Climax Molybdenum Co. Ltd, Imperial House, 1/3 Grosvenor Place, London SW1, UK).

20-24 August, **Artificial Intelligence**, Tokyo (Prof. Raj Reddy, Dept of Computer Science, Carnegie-Mellon University, Pittsburgh, Pennsylvania 15213).

20-25 August, **3rd International Conference on Surface and Colloid Science**, Stockholm (The Swedish Institute for Surface Chemistry, c/o Stockholm Convention Bureau, Strådvagen 7C, S-114 56 Stockholm, Sweden).

26-31 August, **4th International Symposium on Environmental Biogeochemistry**, Canberra (The Conference Secretary, Australia Academy of Science, PO Box 783, Canberra City, ACT 2601, Australia).

28 August-1 September, **Neurochemistry of the Retina**, Athens (Dr Nicolás G. Bazán, Instituto de Investigaciones Bioquímicas, Universidad Nacional del Sur-Conicet, Av Alem 1253, 8000-Bahía Blanca, Argentina).

29-31 August, **IUPAC Symposium on Mycotoxins and Phycotoxins**, Lausanne (Prof. P. Krogh, Dept of Veterinary Microbiology, School of Veterinary Medicine, Purdue University, West Lafayette, Indiana 47907).

30-31 August, **Japan Conference on Polymers and Plastics**, Tokyo (Vijay Mohan Bhatnagar, Alena Enterprises of Canada, PO Box 1779, Cornwall, Ontario, Canada K6H 5V7).

Reports & Publications

UK & Ireland—September

- Birth Impairments. Pp. 60. (London: Office of Health Economics, 162 Regent Street, W1, 1978.) 35p. [19]
- Health and Safety Commission. Health and Safety at Work (Diving Operations) Regulations. (Consultative Document.) Pp. 19. (London: HMSO, 1978.) 50p. net. [19]
- The Radiochemical Centre Limited, Amersham. Seventh Annual Report for the year ended 31st March 1978. Pp. 20. (Amersham: The Radiochemical Centre Limited, 1978.) [19]
- The Universities Central Council on Admissions. Statistical Supplement to the Fifteenth Report, 1976/1977. Pp. 28. (Cheltenham: The Universities Central Council on Admissions, PO Box 28, 1978.) £1.25 [19]
- The Labour Party. Statements to Annual Conference. By the National Executive Committee. Pp. 67. (London: The Labour Party, Smith Square, SW1, 1978.) 45p. [219]
- The Pattern and Range of Services for Problem Drinkers. Report by the Advisory Committee on Alcoholism. Pp. iii+52. (London: Department of Health and Social Security, SH Division, Alexander Fleming House, SE1, 1978.) gratis. [219]
- Royal Commission on the National Health Service. Research Paper No. 4: Doctor Manpower 1975–2008—Alternative Forecasts and their Resource Implications. Pp. xiii+60. (London: HMSO, 1978.) £1.50 net. [219]
- Philosophical Transactions of the Royal Society of London. A: Mathematical and Physical Sciences. Vol. 290, No. 1367: Atmospheric Chemistry—Response to Human Influence. By Jennifer A. Logan, M. J. Prather, S. C. Wofsy and M. B. McElroy. Pp. 187–234. (London: The Royal Society, 1978.) UK £3.15; Overseas £3.30. [229]
- Tetrahedron Reports. No. 41: Bio-mimetic Oxygenation. By Teruo Matsuura. Pp. 39. No. 42: Structure and Reactivity of Alkali Metal Enolates. By Lloyd M. Jackman and Barry C. Lange. Pp. 35. No. 44: Action of l-Hydroxylamine, de l-Hydrazine et de ses Derivés sur les Pyrones. Par Christophe Morin et René Beugelmans. Pp. 13. No. 45: Applications Récentes de la Réaction de Retro-Diels-Alder en Synthèse Organique. Par Jean Ripoll et Annick Rouessac et Francis Rouessac. Pp. 25. No. 46: The Synthesis of Macrocyclic Lactones—Approaches to Complex Macrolide Antibiotics. By Thomas G. Back. Pp. 22. No. 47: The Problem of Conformational Effects. By N. S. Zefirov. Pp. 13. No. 48: Mesomeric Betaine Derivatives of Heteropentanes. By Christopher A. Ramsden. Pp. 35. No. 49: The Biosynthesis of Aromatic Hemiterpenes. By Michael F. Grundon. Pp. 22. (Oxford and New York: Pergamon Press, 1978.) £4.95 each. [229]
- International Union of Pure and Applied Chemistry. Physical Chemistry Division. Recommendations for the Presentation of Infrared Absorption Spectra in Data Collections—A. Condensed Phases. Prepared for publication by E. D. Becker. Pp. 4. £3.30. Recommended Reference Materials for Realization of Physicochemical Properties. Section: Absorbance and Wavelength. Edited by E. F. G. Herington. Pp. 12. £3.30 (Oxford and New York: Pergamon Press, 1978.) [229]
- The Health Effects of Lead on Children: a Review of Literature Published Since 1976. Pp. 28. (Chertsey, Surrey: The Conservation Society, 12a Guildford Street, 1978.) £1. [229]
- Water Research Centre. Annual Report, 1977/1978. Pp. 107. (Henley-on-Thames: Water Research Centre, 45 Station Road, 1978.) [229]
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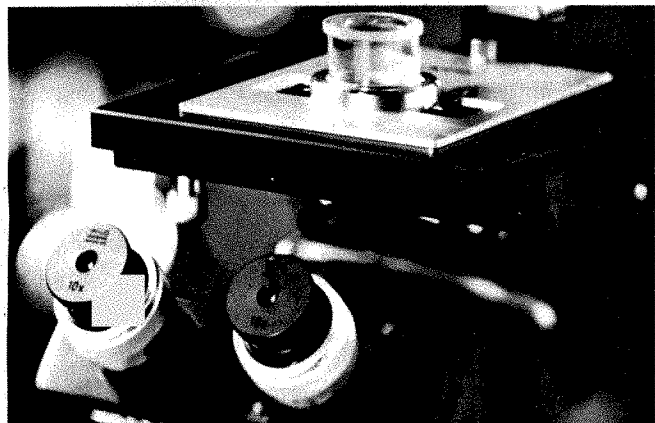
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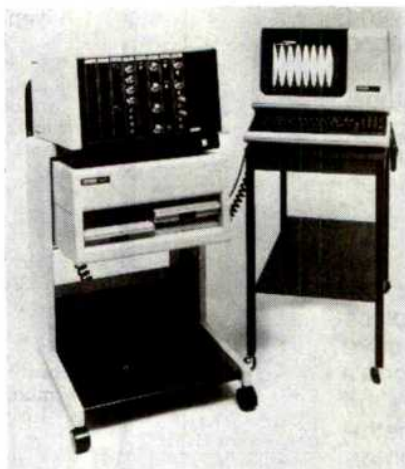
These notes, prepared from material provided by the manufacturers, are intended to give an outline of the range of products on the market. More detailed information may be obtained by circling the appropriate numbers on the Reader Enquiry Card, bound inside the cover.

A feature on biochemicals, enzyme reagents, assay kits and radioimmunoassay will appear in the 14 December issue.

KineticSystems Corporation. KineticSystems announce a new stand-alone microcomputer system for monitoring and control applications as well as countless business and personal uses. The development of this mini CAMAC crate provides proven system performance in a smaller, more economical package. This version of the 8010 Microcomputer System will accommodate a wide variety of needs since it may be programmed in BASIC or assembly language. Because of the modularity of CAMAC, this system is inherently flexible and can be easily configured for nearly any application. The choice of over 1,000 CAMAC modules ensures that each version of an 8010 system provides both an economical solution to process automation and limitless expansion capabilities for future requirements. Communication between the microcomputer and the modular components of the 8010 System is via a microcomputer peripheral bus which supports up to 64 kilobytes of RAM and EPROM memory, diskette drives, and as many as seven crate controllers and their associated crates of process I/O modules. A standard CAMAC crate can contain the 8010 microcomputer system and provide 19 additional stations for monitoring and control modules. The mini CAMAC crate version of the 8010 system offers all the power of the standard configuration in a smaller package containing 12 stations. Two stations are taken by the crate controller, 10 stations are used for I/O modules, and the remaining non-crate area contains two diskette drives with controller, 16 kilobytes of RAM, five kilobytes of EPROM, a microcomputer, and the power supplies. The 8010 System can drive a CAMAC serial highway containing up to 62 remote crates or a CAMAC branch highway containing up to seven crates.

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MINC from Digital Equipment

testing devices to be supported. Also included is a double-density dual diskette unit for program and data storage and a new video graphic terminal, the VT105. The terminal has full alphanumeric capabilities, plus a graphics facility of 512 horizontal by 240 vertical positions. Data can be presented graphically in point or histogram mode, and alphanumerics are presented in upper and lower case, normal, underlined, bold face, and video reverse. Characters can also be presented double-height, double-width, or both.

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7991(A)

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THE UNIVERSITY OF LEEDS

DEPARTMENT OF ORGANIC CHEMISTRY

Applications are invited for two temporary fixed-term posts of POSTDOCTORAL RESEARCH FELLOW. One post will be concerned with the chemistry of trimethylene methane (with Dr R. J. Bushby) and the other post will be for work on the partial synthesis of fluorinated analogues of gibberellin precursors and the study of the effect of these analogues on the biosynthesis of the gibberellins (with Dr B. E. Cross). Both posts are available for a fixed period of one year.

Salary in the range £3,883 to £4,382 of the IA Scale for Research and Analogous Staff (£3,883 to £6,555) (under review).

Applications, giving details of qualifications, research experience and the names and addresses of two referees, should be sent to the Department of Organic Chemistry, The University of Leeds, Leeds LS2 9JT, as soon as possible. 7993(A)

LINCOLN COLLEGE New Zealand

CHAIR IN ENTOMOLOGY (Agricultural Zoology)

The Council of Lincoln College, University College of Agriculture, invites applications for the Chair in Entomology, resulting from the impending retirement of Professor R. A. Harrison.

Applicants should hold an advanced degree in Science or Agricultural Science and have an established record of research and/or teaching in some branch of agricultural zoology.

The successful applicant will be appointed as Head of Department and will be responsible to the College Principal for the administration of his Department.

The appointment will be made within the existing range of professorial salaries, NZ\$23,012 to NZ\$29,188 per annum, the commencing salary being determined by the College Council.

Expenses of appointment reimbursed up to specified limits. New Zealand Government Superannuation is available.

Further details and general Conditions of Appointment are obtainable from the Secretary General, Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on January 15, 1979. 8041(A)

COMMONWEALTH AGRICULTURAL BUREAUX

VACANCY FOR SECRETARY TO EXECUTIVE COUNCIL

at

Farnham House, Farnham Royal, Slough SL2 3BN

Duties—The Secretary will be responsible to the Executive Director for the secretarial, administrative, personnel and accounting functions.

Qualifications—Graduate candidates not over 50 will be preferred. Experience in accountancy and administration at a senior level is essential, and a knowledge of law and practices affecting employment would be an advantage.

Salary in the scale £7,825 to £8,729 with provision for superannuation. Initial salary according to age, qualifications and experience.

Applications—Full particulars may be obtained from the Executive Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal, Slough SL2 3BN. Completed forms should be returned to the Executive Director not later than November 30, 1978. 7996(A)

RESEARCH TECHNICIAN GRADE 3

required to assist with project investigating rabbit red blood cell development, with particular reference to M.R.N.A. and protein synthesis. Knowledge of protein fractionation, chromatography, centrifugation and radioactive techniques is desirable. Minimum qualifications O.N.C./H.N.C. or equivalent, with at least 3 years relevant experience. The post is financed by the M.R.C. for 3 years in the first instance. Salary on scale £3,153 p.a. rising to £3,525 p.a. inclusive (under review). Four weeks annual holiday. College contributory pension scheme. Apply in writing with full details to: The Head Clerk Ref. 204019/N, King's College London, Strand WC2R 2LS. 8042(A)

AUSTRALIAN NATIONAL UNIVERSITY

Applications are invited for appointment to the following:

Faculty of Science

DEPARTMENT OF BOTANY

SENIOR LECTURER IN GENETICS

Applicants for this position must have broad interests in and knowledge of genetics, substantial experience in University teaching, and a record of productive research. Appointment as Senior Lecturer is for three years in the first instance with possibility of reappointment, after review, to retiring age.

Salary on appointment will be in accordance with qualifications and experience within the range \$A20,365 to \$23,737 per annum. Current exchange rates \$A1 = \$US1.15; £0.58p.

CLOSING DATE: December 2, 1978

Other conditions: Reasonable appointment expenses are paid. Superannuation benefits are available for applicants who are eligible to contribute. Assistance with housing is provided for an appointee from outside Canberra.

The University reserves the right to make an appointment or to make an appointment by invitation at any time.

Prospective applicants should obtain the further particulars from the Association of Commonwealth Universities (Apts), 36 Gordon Square, London WC1H 0PF. 8040(A)

UNIVERSITY OF SURREY

DEPARTMENT OF BIOCHEMISTRY

RESEARCH OFFICER— CANCER RESEARCH

Applications are invited for the post of Research Officer to undertake research into factors affecting the development of lung cancer. Preference will be given to applicants with experience in at least one of the following areas: Biochemistry of nucleic acids, tumour promoters and handling of animals. The work is supported by the Medical Research Council and the position is tenable for two years. Salary: £3,384 to £3,883.

Applications, in the form of a curriculum vitae, and the names and addresses of two referees should be sent to the Staff Officer, University of Surrey, Guildford, Surrey GU2 5XH by November 10, 1978.

Further particulars may be obtained from Dr G. M. Cohen, Department of Biochemistry, University of Surrey, or telephone Guildford 71281, ext. 821. 8006(A)

UNIVERSITY OF SOUTHAMPTON

FACULTY OF MATHEMATICAL STUDIES CHAIR OF OPERATIONAL RESEARCH

Applications are invited for the Chair of Operational Research. Salary within the professorial range. Applicants should have an established reputation in the field of Operational Research. Further particulars may be obtained from the Secretary and Registrar, The University, Southampton SO9 5NH, and applications (11 copies from applicants in the United Kingdom) must be submitted before November 28, 1978. Please quote ref. MA2/1/Na. 8022(A)

CHANGE OF ADDRESS

The Advertisement offices for NATURE have moved to
3 DYERS BUILDINGS, LONDON, EC1N 2NR
TELEPHONE: 01 831-6901

Research in Forensic Science

Development of analytical techniques, instrumentation and data handling

The Central Research Establishment of the Home Office at Aldermaston, is actively engaging in the overall research effort and co-ordination of the work within the Forensic Science Service, while also acting as an information centre for the Service as a whole. The Establishment, equipped with the most modern and sophisticated techniques to analyse and examine a wide range of materials, currently has a number of challenging career opportunities for Research Scientists within four operational Divisions:

BIOLOGY

Opportunities for Biochemists and Serologists to research new methods of grouping human tissues, including saliva, semen and hair. There is a particular need for a Serologist with wide experience of hospital pathology laboratory or blood transfusion work, and for a Biochemist with knowledge of protein fractionation techniques (eg electrophoresis).

TOXICOLOGY

Analytical Chemists and Biochemists are needed to research new methods of identification and quantification for drugs and metabolites in body tissues

and fluids using HPLC, GLC, MS and RIA techniques. Experience in the fields of pharmacokinetics and drug metabolism would be an advantage.

CHEMISTRY

Chemists and Physicists are required for general trace analysis of materials of all kinds using XRF, IPC-Arc, Pyrolysis Mass Spectrometry, AAS, UV/IR Spectrophotometry together with computerised data storage, etc.

INFORMATION

This division needs Information Scientists, Chemists and Biologists for the development of data acquisition, storage and dissemination procedures for use on the Division's own computer and terminal facilities with on-line links to the Regional Forensic Science Laboratories, also for operating Quality Assurance and External Contract procedures and for answering urgent requests for information from the regional laboratories.

INFORMATION (COMPUTER SECTION)

A further vacancy exists for a Computer Scientist to work on the day-to-day operations of a 16K Hewlett-Packard

21000A computer and associated peripheral equipment, also involving maintenance of the equipment and programming of new applications in forensic science. A knowledge of small computer systems and Fortran is essential.

The above opportunities are available at Scientific Officer, Higher Scientific Officer and Senior Scientific Officer levels for which a relevant honours degree is preferable. Salary levels according to age and experience: SO (£2,840-£4,410); HSO (£4,100-£5,450) at least 2 years postgraduate experience required; SSO (£5,150-£6,900) at least 4 years postgraduate experience. Good promotion prospects. Non-contributory pension scheme.

For an application form (to be returned by 10 November, 1978) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote ref: SB/20/LA.

**Science
Group**
CIVIL SERVICE

Home Office Central Research Establishment

TECHNICIAN CELL BIOLOGY

Division of Viral Products requires a suitably qualified laboratory technician to work in the field of cell biology.

Preference will be given to candidates (male/female) with experience in tissue culture techniques of mammalian cells and/or biochemical techniques applied to virology.

Salary—dependent on age, qualifications and experience on scale £3,815 to £5,034 p.a.

Applications and further details from Personnel Officer, NIBSC, Holly Hill, Hampstead, NW3 6RB Tel: 01-435 2232 ext 211 quoting reference number 0170.

NIBSC

National Institute for Biological Standards and Control
9016(A)

HEAD OF PHARMACOLOGY DEPARTMENT

A leading Spanish Pharmaceutical Company situated in the mediterranean city of Barcelona invites applications from pharmacologists for the above post in a Pharmacology Department. The man we appoint will have a good honours degree in an appropriate discipline, a Ph.D. in pharmacology and several years postdoctoral experience preferably in the pharmaceutical industry.

The successful applicant will be responsible to the director of Biological Sciences and must be able to formulate and organise research and development projects and collaborate with the other departments in the Biological and Chemical science groups.

Knowledge of Spanish is not initially essential but the successful candidate must be prepared to acquire adequate fluency in this language within 2 years. The salary is tax free and negotiable at around 30,000 dollars p.a. and special assistance will be given for removal expenses to Barcelona.

Applications in writing with full curriculum vitae should be addressed to:

Pharmacology Department
P.O.B. 1583
BARCELONA-24 (Spain) 7986(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the temporary post of

DEMONSTRATOR

In the above Department from suitably qualified candidates. The person appointed will participate in teaching biochemistry to science and to medical students and will also be expected to carry out research on the molecular biology of bacterial drug resistance in collaboration with Dr J. H. Parish. The post is tenable from January 1, 1979 until September 30, 1979. Salary at an appropriate point on the IB Scale for Other Related Staff (£3,384 to £5,604) (under review) according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University of Leeds, Leeds LS2 9JT, quoting reference number 83/16/D. Closing date for applications November 6, 1978 8002(A)

UNIVERSITY OF SALFORD

DEPARTMENT OF BIOLOGY

POSTDOCTORAL RESEARCH FELLOW

Applications are invited for a Postdoctoral Research Fellow sponsored by the Science Research Council, to investigate the respiratory physiology of birds during exercise (non-flying) and vocalisation under the supervision of Dr J. H. Brackenbury. Experience in respiratory/blood gas analysis an advantage. The appointment is tenable for three years commencing as soon as possible.

Commencing salary within the range £3,883 to £4,328 per annum. Application forms obtainable from the Registrar, University of Salford, Salford M5 4WT (tel: 061-736 5843, ext. 215) to whom completed applications should be returned not later than Friday, November 17, 1978, quoting reference number B/83/N 8064(A)

THE ROYAL VETERINARY COLLEGE

University of London
Division of Preclinical Studies
DEPARTMENT OF
PHYSIOLOGY
LECTURER IN
PHARMACOLOGY

Applications are invited from veterinary, medical and science graduates, including recent veterinary graduates who wish to make a career in Veterinary Pharmacology. The main teaching responsibility is Veterinary Pharmacology for the BVetMed degree and it is also closely linked with the teaching of therapeutics. The appointee will also be expected to assist with the Honours B.Sc. course, to pursue research and to advance knowledge of the scientific basis of veterinary therapeutics.

Prospective applicants are encouraged to get in touch with either the Head of Department, Professor M. G. M. Jukes, or Dr P. Lees, Reader in Veterinary Pharmacology.

SALARY SCALE (under review). £4,333 to £5,204 including London Allowance. Initial salary determined by experience and qualifications. Superannuation under the Universities' scheme.

The post is available from a date to be arranged.

Removal expenses up to £800 may be reimbursed in approved circumstances.

Application form and further details from the Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU (telephone 01-357 2896).

CLOSING DATE FOR APPLICATIONS: November 10, 1978 7948(A)

QUEEN MARY COLLEGE

University of London

Applications are invited for the post of MOLECULAR GENETICIST to undertake research on the genetic manipulation of yeast with special reference to transformation studies. Applicants should have research experience in Microbial Genetics and/or Molecular Biology.

The appointment is sponsored by a major industrial organisation and the selected candidate will be on the payroll of the Company concerned. Following a 2-3 year period of research at Queen Mary College, the successful candidate will be transferred to the Company's own research laboratories.

A competitive salary and non-contributory pension rights in accordance with the Company's own scale will be offered to the successful candidate. Further details will be given at interview.

Applications should be made by October 27 to: Professor E. A. Bevan (N), Professor of Genetics, Queen Mary College, Mile End Road, London E1 4NS. 8039(A)

Please mention

Nature

when replying to
these advertisements

THE OPEN UNIVERSITY LECTURERS IN BIOLOGY



We have two vacancies: those appointed will be involved in the preparation and maintenance of the discipline's 2nd and 3rd level courses in one or more of the areas of Ecology, Mammalian Physiology, and Molecular Biology/Biochemistry. They will also be expected to take an active interest in the development of the discipline's future offerings in the field of continuing education, and to undertake research—either independently, or with one of the existing research groups. Successful candidates should have a good research record and teaching experience.

Applications for the posts should be able to demonstrate an ability to write clearly and easily and an interest in distance teaching. Both posts are tenured. Salary will be on the scale £3,883-£7,764 (under review) and there are USS and other benefits.

Application forms and further particulars are available, by postcard request please, from the Recruitment Office (JD3682/4), The Open University, PO Box 75, Walton Hall, Milton Keynes, MK7 6AL, or telephone Milton Keynes 63868. 24 hour answering service.

Closing date for applications: 3rd November, 1978 8064(A)

QUEEN CHARLOTTE'S MATERNITY HOSPITAL

Goldhawk Road, London W6

BASIC GRADE or SCIENTIFIC OFFICER (Cytogenetics)

Applications are invited from science graduates with honours degrees for the above posts. Preference will be given to candidates with experience in diagnostic work in human cytogenetics. Whitley Council terms and conditions of service. The diagnostic laboratory which is fully equipped, may be visited by appointment. Interested applicants are advised to contact Dr D. Huxley (Tel: 01-748 4666, Ext. 223) in the first instance.

Application forms obtainable from and returnable to The Administrative Assistant (Ext: 204) 8014(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF PHYSICS RESEARCH ASSOCIATE

Applications are invited for a postdoctoral Research Associate to undertake a study of liquid semiconductor alloys using photoelectron spectroscopy. The project is part of a wide investigation of the properties of disordered systems being carried out by the Physics Department and supported by the Science Research Council.

The post is tenable for up to 2 years and the salary is from £3,883 p.a.

Applications with a curriculum vitae and the names of two referees should be sent to Dr C. Norris, Department of Physics, University of Leicester, LE1 7RH. 8049(A)

CHANGE OF ADDRESS

The Advertisement offices for NATURE have moved to
3 DYERS BUILDINGS, LONDON, EC1N 2NR
TELEPHONE: 01 831-6901

THE OPEN UNIVERSITY

Faculty of Science Research Assistant

Applications are invited for a post of Research Assistant in the Science Faculty. The post is funded by a research grant and is available for two years.

An analysis of the educational effectiveness of the Open University Science Foundation Course has been undertaken to enable an improved version of the Course to be made and presented to students in 1979. Information will be collected on the educational effectiveness of the new Course and the Research Assistant will be required to assist in the collection and analysis of this material.

Candidates should have a first degree in a scientific subject and an interest in educational technology is essential. Familiarity with Open University courses, particularly S100 would be an advantage.

Application forms and further particulars are available by postcard request please from The Recruitment Office (JD2891/4), The Open University, P.O. Box 76, Walton Hall, Milton Keynes, MK7 6AL, or telephone Milton Keynes 63404: there is a 24 hour answering service on 63868.

Closing date for applications: 3rd November 1978. 8043(A)



THE UNIVERSITY OF SHEFFIELD

DEPARTMENT OF PHYSICS

Applications are invited from men and women for a RESEARCH POST in the field of MAGNETOSPHERIC PHYSICS, financed by the S.R.C. and tenable for three years. The person appointed will assist in the analysis of V.L.F. wave data from the GEOS 1 and 2 satellites as part of a collaborative European programme. Candidates should have postgraduate experience in an appropriate field and computing experience is highly desirable. Salary on Range 1A up to £4,382 per annum. Particulars from the Registrar and Secretary, the University, Sheffield S10 2TN, to whom applications should be sent together with the names of two referees as soon as possible. Quote ref: R 207/G. 8001(A)

UNIVERSITY COLLEGE HOSPITAL

DEPARTMENT OF MEDICAL PHYSICS PHYSICIST/ELECTRONICS ENGINEER

(Basic Grade Physicist Scale)
MEDICAL LABORATORY
SCIENTIFIC OFFICER

Two vacancies exist in a team involved in the development and evaluation of blood gas analysis sensors. The Physicist/Electronics Engineer will be expected to collaborate on the design and building of instrumentation associated with the sensors. The M.L.S.O. will be involved in the laboratory testing of the sensors together with animal and clinical trials.

Both appointments are for one year in the first instance.

Applications with the names of two referees should be submitted to, Mr D. Delpy, Dept. of Medical Physics, U.C.H., Shropshire House, Cannon Street, London W.C.1. Tel. 387 9300 Ext. 119. 8038(A)

The Department of Zoology of the University of California, Davis announces the vacancy of an ASSISTANT PROFESSORSHIP in the area of Developmental Biology

The Department of Zoology invites applications from persons with primary interests and experience in research and teaching in any area of developmental biology. The appointee is expected to establish a productive research program and to participate in the department's program of graduate instruction. Teaching responsibilities include a one-quarter introductory course in embryology, a one-quarter advanced course in developmental biology, and participation in a graduate seminar in developmental biology. Candidates must have a Ph.D. degree. Salary will depend upon qualifications and experience.

Applicants should forward a curriculum vitae, a description of their research experience and future research plans, reprints of all publications, a summary of teaching capabilities, and three letters of recommendation to Dr R. D. Grey, Chairperson of the Search Committee, Department of Zoology, University of California, Davis, CA 95616. The University of California is an equal opportunity/affirmative action employer. Closing date for receipt of applications is November 30, 1978. 7975(A)

SCHERING erwartet Sie

Für unser Department Entzündungspharmakologie, mit den Arbeitsgruppen Dermatologie, Rheumatologie, Allergologie, suchen wir einen

Mediziner oder Biologen

mit mehrjähriger experimenteller Erfahrung. Unser neuer Mitarbeiter soll die Arbeitsgruppe Rheumatologie leiten. Er wird an der Wirkstofffindung teilnehmen und das Gebiet in seinem vorklinisch experimentellen Teil im Hause und extern vertreten. Der Schwerpunkt seiner wissenschaftlichen Arbeiten wird auf dem Gebiet der Immunologie und Molekularbiologie liegen.

Wenn Sie dieses Arbeitsgebiet interessiert würden wir uns freuen, mit Ihnen Kontakt aufnehmen zu können und mit Ihnen zu diskutieren. Bitte, schicken Sie uns Ihre Bewerbungsunterlagen oder rufen Sie uns an (Tel. 030/468 2853 oder 468 2922).

SCHERING AG, Personalabteilung Berlin
Müllerstraße 170-178, 1000 Berlin 65

SCHERING AG

8006(A)

KENYATTA UNIVERSITY COLLEGE—KENYA

a constituent college of the University of Nairobi

Applications are invited for the post of ASSOCIATE PROFESSOR in the

DEPARTMENT OF CHEMISTRY

Candidates must have a Ph.D. in Chemistry and considerable teaching experience at University level, at least a Senior Lecturer level. Further qualifications in Education or experience in the training of graduate teachers would be advantageous. The appointee will be expected to teach in the B.Ed. and A.Sc. programmes and to carry out and supervise research in his/her areas of specialisation. He/she will also be expected to be capable of providing academic and administrative leadership in the department.

Salary scale: K£3,864 to K£4,488 p.a. (£21=£1.34 sterling). The British Government may supplement salary by 5,052 p.a. (sterling) for married appointee or £3,438 p.a. (sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages, subsidised housing, S.S.F. or F.S.S.U.; non-contributory medical and scheme. Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to the Registrar, Kenyatta University College, P.O. Box 3844, Nairobi, Kenya, by November 6, 1978.

Applicants resident in the U.K. should also send one copy to Inter University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from this address. 8046(A)

SUNY at BUFFALO seeks applications and nominations for the post of CHAIRMAN, MATHEMATICS

Leadership of about 40 faculty including applied mathematics—the Departments of Computer Science and of Statistics are separate. SUNY/B is the largest of the four University Centers of the State University of New York, with about 25,000 students including about 80 math graduate students.

Principal strengths of the Department are in algebra, analysis, applied mathematics, and logic.

Vitae and names of two or three referees should be sent to Professor H. Segal, 109 Cooke Hall, SUNY/B, Amherst, NY 14260, by January 10, 1979.

SUNY/B is an affirmative action/equal opportunity employer 8023(A)

CHANGE OF ADDRESS

The Advertisement offices for NATURE have moved to
3 DYERS BUILDINGS, LONDON, EC1N 2NR
TELEPHONE: 01 831-6901

UNIVERSITY OF BASEL FACULTY OF SCIENCE

In connection with the reorganisation of the Department of Zoology applications are invited for the following positions:

PROFESSOR from October 1, 1979

ASSOCIATE PROFESSOR from
November 1, 1979

A further Professor is expected to be appointed at a later date.

Experience is desired in one of the following fields: Sensory physiology, ecological ethology, morphology including electron microscopy.

Duties include general teaching in zoology and collaboration in research and teaching with special emphasis on ecological aspects.

Applications stating publications, teaching experience, curriculum vitae etc. should be submitted until the end of December 1978.

Address: Dekanat der Philosophisch-Naturwissenschaftlichen Fakultät der Universität Basel, Klingelbergstrasse 70,
CH-4056 BASEL / Switzerland.
7992(A)

ANATOMIST

Applications are invited for a vacant position in Anatomy at the assistant or associate professor level. Applicants should possess an M.D. and/or Ph.D., and have experience in teaching gross anatomy to medical students. Experience in teaching embryology or histology would be an asset.

Candidates would be expected to have a strong research background. Excellent facilities are available in histology, neuroscience, molecular biology, immunology and cell physiology.

Salary will be commensurate with experience.

Applicants should send a curriculum vitae and names of three referees to Dr T. M. Scott, Chairman Anatomy Search Committee, Faculty of Medicine, Health Sciences Center, Memorial University, St. John's, Newfoundland, Canada A1B 3V6.

8047(A)

ROYAL MARSDEN HOSPITAL

Downs Road, Sutton, Surrey
MEDICAL LABORATORY
SCIENTIFIC OFFICER—
CLINICAL PATHOLOGY

Required for Clinical Chemistry section of Clinical Pathology Department. Experience and/or interest in protein analysis would be useful.

Application forms from Personnel Department R.M.H. London tel: 352 1171 Ext. 447.

Closing date: October 31, 1978.

8011(A)

UNIVERSITY OF EXETER DEPARTMENT OF BIOLOGICAL SCIENCES RESEARCH TECHNICIAN

required to assist in a project supported by the S.R.C., to investigate the mechanism of cytoplasmic transport in cells. Previous experience in biochemical techniques and microscopy would be an advantage.

The appointment will be for two years, starting as soon as possible, with a salary £2,688 to £3,060 per annum (Grade III). Minimum qualifications: O.N.C. Science and preferably three years' experience.

Applications giving full details and naming two referees should be sent to Dr H. Stebbings, Washington-Singer Laboratories, Perry Road, Exeter EX4 4QG, to reach him not later than November 3, 1978.

8035(A)

QUEEN'S UNIVERSITY, Kingston, Canada. Plant Scientist. Research and teaching position for a scientist with an established research program, preferably in the area of plant physiology. Rank and salary are negotiable but are expected to be in the middle range. Those interested should send a detailed resume together with the names of three referees to H. M. Good, Chairman Selection Committee, Department of Biology, Queen's University, Kingston, Ontario, Canada.
7984(A)

AGRICULTURAL COLLEGE OF ATHENS

1. The Agricultural College of Athens, Greece, a University level Institution, has a vacancy for the Chair in

FLORICULTURE AND LANDSCAPE ARCHITECTURE

This position will be officially advertised within 1978 and it is open for qualified candidates who have Greek citizenship with a Ph.D. or equivalent recognised degree in Ornamental and/or Environmental Horticulture. Interested specialists should submit as soon as possible a letter with a biographical sketch to the Dean, Professor D. Papamichael, Agricultural College of Athens, Botanikos, Athens 301, Greece. Details for application will be forwarded upon request.

2. The Agricultural College of Athens, Greece, a University level Institution, has a vacancy for the Chair in

VEGETABLE CROPS

This position will be officially advertised within 1978 and it is open for qualified candidates who have Greek citizenship with a Ph.D. or equivalent recognised degree in Vegetable Crops. Interested specialists should submit as soon as possible a letter with a biographical sketch to the Dean, Professor D. Papamichael, Agricultural College of Athens, Botanikos, Athens 301, Greece. Details for application will be forwarded upon request.

7985(A)

UNIVERSITY OF NAIROBI KENYA

Applications are invited for the post of **SENIOR LECTURER IN THE DEPARTMENT OF ANIMAL PHYSIOLOGY**. Applicants must have Ph.D. in mammalian physiology, preferably in ruminant gastroenterology, neurophysiology or cardiovascular physiology. They must have considerable teaching experience at University level. Applications should be accompanied by six reprints of the candidate's most important papers as evidence of ability to organise and carry out meaningful and original research. Salary Scale: K£2,988 to K£3,984 p.a. (K£1-£1.35 Sterling). The British Government may supplement salary by £4,770 p.a. (Sterling) for married appointee or £3,252 p.a. (Sterling) for single appointee (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; S.S.S.F. or F.S.S.U.; subsidised housing/housing allowance; non-contributory medical aid scheme. Detailed applications (2 copies) with curriculum vitae and naming 3 referees to be sent direct to Registrar, Recruitment and Training, University of Nairobi, P.O. Box 30197, Nairobi, Kenya by November 20, 1978. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

8037(A)

BIOCHEMIST, Probationary Grade, Whitley A Scales, required for the routine department of Chemical Pathology, 17 Page Street, London SW1P 2AR. Possibility of London M.Sc. course to follow, with excellent training rotation. Applicants with 1st or 2nd Class Honours should write to Professor J. R. Hobbs at the above Department, with the names of two referees, by October 30, 1978.

8027(A)

UNIVERSITY COLLEGE OF NORTH WALES/GWYNEDD HEALTH AUTHORITY SENIOR RESEARCH OFFICER IN ULTRASOUND

Applications are invited for this new joint appointment which provides a challenging opportunity to develop a research programme in ultrasound at the College, and to give scientific support to an evolving clinical ultrasound service for North Wales.

Applicants should have a good Honours degree or similar qualification, proven research ability and relevant experience. The appointment will be to the Universities' Research and Analogous grades 1A/II, £3,883 to £7,754 per annum.

Applications (two copies), together with the names and addresses of three referees, should be sent to the Assistant Registrar (Personnel), University College of North Wales, Bangor, Gwynedd, LL57 2DG, from whom further particulars may be obtained.

Closing date for applications: October 31, 1978. 7974(A)

ASSOCIATE RESEARCH POSITION

Applications are invited from post-doctoral fellows with the experience and the background involved in the isolation and cloning of gene sequences. Salary commensurate with experience. Knowledge of German is not required.

Forward letter of application giving full details and the names and addresses of two referees to:

Dr A. Ruiz-Carrillo, Max-Planck-Institut für Molekulare Genetik 1000 Berlin 33, Ihnestrasse 63-73, BRD.
7979(A)

VICTORIAN COLLEGE OF PHARMACY LTD Parkville, Victoria, Australia DEAN SCHOOL OF PHARMACEUTICAL CHEMISTRY

(Salary Aust.\$31,789 p.a.)

(£518,536 at September 26, 1978 ex. rate)

Applications are invited for the above position which will become vacant on January 1, 1979 when the present holder, Dr G. N. Vaughan takes up the position of Dean of the College.

The College is divided into three semi-autonomous Schools: Pharmaceutical Chemistry, Pharmacology and Pharmaceutical Chemistry. The present Deans of Schools are Professor I. H. Pitman (Pharmaceutics) and Professor C. Raper (Pharmacology).

A full description of the College, the School of Pharmaceutical Chemistry and the specifications for the position are available from Mr R. Burnet, Registrar, Victorian College of Pharmacy Ltd., 381 Royal Parade, Parkville, Victoria, Australia, 3052. (telephone (03) 380 6254).

Preference will be given to applicants of proven strength in Medicinal Chemistry, but any person who has had experience in pharmacy and pharmaceutical education in any area related to analytical chemistry, physical chemistry, biological chemistry, etc., could be considered as suitable applicants. The position commands full professional status.

Applications close December 31, 1978.

8017(A)

CHANGE OF ADDRESS

The Advertisement offices for NATURE have moved to
3 DYERS BUILDINGS, LONDON, EC1N 2NR
TELEPHONE : 01 831-6901

ANIMAL HEALTH TRUST

Small Animals Centre

MICROBIOLOGIST

We are expanding our microbiological facilities and wish to appoint microbiologist or bacteriologist to supervise the running of a laboratory which services internal research projects and provides a routine service to veterinary surgeons in practice. Current topics of research interest include scarlet diseases of infectious origin, especially infectious bovine keratoconjunctivitis, canine renal disease and the fading puppy complex. Applicants should possess a good honours degree in a relevant discipline. Veterinary surgeons are welcome to apply and would be expected to specialise in one of the above fields. The successful applicant would be encouraged to register for a higher degree. Salary commensurate with University Scales.

The laboratories are modern, well equipped and situated four miles from Newmarket in pleasant parkland. Further details of the appointment can be obtained from the Director, Animal Health Trust, Small Animals Centre, Lanwades Park, Kennett, Nr. Newmarket, Suffolk CB8 7PN.

7981(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the post-doctoral post of Senior Research Assistant to work on a project funded by a Cancer Research Campaign grant to Dr A. G. McLennan on the use of viruses as probes for error-prone DNA repair in human fibroblasts. Experience of tissue culture and nucleic acid techniques desirable. The appointment is for one year in the first instance and may be renewed for a further two years. Initial salary within the range £3,384 to £4,382 per annum.

Applications together with the names of two referees, should be received not later than November 3, 1978, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/965/N.

7982(A)

UNIVERSITY OF GLASGOW

DEPARTMENT OF BIOCHEMISTRY

POSTDOCTORAL RESEARCH ASSISTANT

Applications are invited for a Post-doctoral Research Assistant supported by the M.R.C. to work with Dr B. E. H. Maden on the fine structure of the ribosomal genes in vertebrates. The work will involve recombinant DNA technology and is part of a group project aimed at the molecular characterisation of RNA processing events during ribosome maturation. The appointment will commence as soon as possible and will continue until September 14, 1981, subject to satisfactory progress.

The salary will commence within the range £3,883 to £5,129 on Range 1A of the salary scale for Research and Analogous Staff, the starting point to be determined by the successful candidate's age, qualifications and experience.

Applications, including curriculum vitae and the names of two referees, should be sent to Dr B. E. H. Maden, Department of Biochemistry, University of Glasgow, Glasgow G12 8QQ. In reply please quote Ref. No. 1261M.

8052(A)

KENYATTA UNIVERSITY COLLEGE—KENYA

(a constituent college of the University of Nairobi)

Applications are invited for the following posts in the DEPARTMENT OF GEOGRAPHY:—

ASSOCIATE PROFESSOR. Candidates should hold a Ph.D. in Geography and considerable teaching experience at University level, preferably at Senior Lecturer level. They should also provide evidence of successful research activity through publications in respectable geographical and allied journals. The appointee will be expected to have experience in training of secondary school teachers and to be capable of providing academic and administrative leadership in the department.

SENIOR LECTURER. Applicants should hold a Ph.D. in Geography plus substantial teaching and research experience at University level. All fields of specialisation will be considered but possession of special qualifications in Human Geography will be advantageous.

Salary scales: Associate Professor K£3,864 to K£4,488 per annum, Senior Lecturer K£2,988 to K£3,984 per annum (K£1 equals £1.34 sterling). The British Government may supplement salaries in range £4,770 to £5,052 per annum (sterling) for married appointees and £3,252 to £3,438 per annum (sterling) for single appointees (reviewed annually and normally free of all tax) and provide children's education allowances and holiday visit passages. Family passages; subsidized housing; S.S.S.F. or F.S.S.U.; non-contributory medical aid scheme. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent direct to the Registrar, Kenyatta University College, P.O. Box 48344, Nairobi, Kenya by November 16, 1978. Applicants resident in the U.K. should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address.

8020(A)

ELECTRON MICROSCOPE TECHNICIAN required to work in a modern laboratory on a research programme supported by the Cancer Research Campaign. Appointment for two years in the first instance. Applicants should have experience in histology and electron microscopy on biological material. Preference given to applicants with the I.S.T. Diploma of Electron Microscopy. Salary on Whitley Council Scale. Applications with curriculum vitae and names of two referees by October 31, to Dr R. M. Hicks, School of Pathology, The Middlesex Hospital Medical School, London W1P 7LD.

8019(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF PHYSICS

Applications are invited for the post of Lecturer in the Department of Physics. Candidates should have experience in experimental nuclear structure physics and will be expected to carry out their research at the Nuclear Structure Facility at Daresbury.

Initial salary within the range £3,883 to £5,367 per annum on a scale rising to £7,754 per annum.

Applications, together with the names of three referees should be received not later than November 29, 1978 by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/967/N.

8030(A)

Ministry of Agriculture,
Fisheries and Food, Plant Pathology
Laboratory, Harpenden

Agriculturist/ Horticulturist/ Biologist

■ Make field surveys and visit farmers to determine the extent and methods of use of pesticides ■ Process data by computer.

□ Degree, HNC or equivalent in agricultural, horticultural or biological subject □ Experience in computing advantageous □ Possession of current driving licence essential □ Age normally under 27 □ Appointment as Scientific Officer (£2840-£4410) □ Ref: SB/18/AG.

■ Application forms (to be returned by 13 November 1978) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke (0256) 68551 (answering service if office closed).

Safety in the Food & Cosmetic Industries

Information Scientist

... to work on problems associated with the safety in use of cosmetics, food-contact materials and medical devices. The work includes scanning literature for relevant articles for reference files, carrying out literature surveys on various chemicals associated with cosmetics and food-contact materials, preparing reports and papers for consideration by expert advisory committees, collating and evaluating data, and consulting with representatives of the cosmetic and food industries.

Candidates, normally aged under 30, should have a degree or equivalent qualification in a relevant discipline and several years postgraduate experience. A knowledge of toxicology would be an advantage.

Appointment as Higher Scientific Officer (£4560-£5910). Starting salary according to qualifications and experience. Non-contributory pension scheme. Promotion prospects.

For further details and an application form (to be returned by 9 November 1978) write to Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, or telephone Basingstoke (0256) 68551 (answering service operates outside office hours). Please quote ref: SB/3/KE.

Department of Health & Social Security

8048(A)

Science Group
CIVIL SERVICE

CHANGE OF ADDRESS

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3 DYERS BUILDINGS, LONDON, EC1N 2NR
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THE UNIVERSITY OF BRITISH COLUMBIA

DEPARTMENT OF
CHEMISTRY

Applications are invited from candidates with teaching and research interest in any aspect of IN-ORGANIC CHEMISTRY including BIOLOGICAL INORGANIC CHEMISTRY for an appointment at the Assistant Professorship level.

Applications consisting of a curriculum vitae, a list of publications and the names and addresses of three referees should be sent to the undersigned before December 31, 1978. This post is open to both male and female applicants.

Professor Charles A. McDowell
Department of Chemistry
University of British Columbia
2075 Wesbrook Mall
Vancouver, British Columbia
Canada V6T 1W5

8032(A)

THE ROWETT RESEARCH INSTITUTE

Buckburn, Aberdeen, AB2 9SB

BIOCHEMIST

SCIENTIFIC OFFICER/HIGHER SCIENTIFIC OFFICER

A Biochemist is required to investigate the relationship between cell wall structure and digestibility of forages. This post is in the Carbohydrate Biochemistry Department (Head: Dr J. S. D. Bacon), which has as its main interest the digestion of polysaccharides by ruminants. Members of the Department have wide experience in the determination of polysaccharide structure and in the purification and characterisation of enzymes attacking the glycosidic bond, and the Department is well equipped in these respects.

The successful candidate will work on the chemical characterisation of the factors that limit the digestion of the cell walls of forages by the microbial flora of the rumen, entailing studies on the preparation of cell walls, their composition, the kinetics of their degradation and the morphological changes associated with the microbiological processes.

The results are expected to throw light on the structural features of the plant cell wall that affect its digestibility and also upon the chemical treatments that may increase it.

Qualifications: First or upper second class Honours Degree in Biochemistry, and an interest in Agriculture and Animal Nutrition.

Salary Scales: Scientific Officer £3,294 to £4,415 per annum or Higher Scientific Officer £4,101 to £5,448. Starting salary and grade will be dependent on experience. At least two years' relevant post graduate experience is required for consideration for appointment in the HSO grade.

There is a non-contributory Superannuation Scheme.

Applications, together with a curriculum vitae and the names and addresses of two referees should be sent to the Secretary of the Institute, from whom further particulars can be obtained.

Closing date November 10, 1978

8026(A)

THE ROYAL
VETERINARY COLLEGE

University of London

Division of Paraclinical Studies

DEPARTMENT OF PATHOLOGY

LECTURER IN

VETERINARY PATHOLOGY

Applications are invited from veterinary graduates for the post of Lecturer in Veterinary Pathology. Duties will include participation in teaching the undergraduate and post-graduate courses in pathology and some diagnostic work. The successful applicant will be encouraged to take advantage of the good research opportunities which are available.

Preference will be given to applicants having some relevant experience in postmortem diagnosis, histopathology or similar fields, but consideration will be given to applications from recent graduates or others.

SALARY SCALE (UNDER REVIEW). £4,333 to £8,204 inclusive of London Allowance. Initial salary will be determined according to qualifications and experience. Superannuation under the Universities' scheme.

Removal expenses up to £800 may be reimbursed in approved circumstances.

Application form and further details from the Assistant Secretary (Personnel), The Royal Veterinary College, Royal College Street, London NW1 0TU. Telephone: 01-357 2898.

CLOSING DATE FOR APPLICATIONS: November 6, 1978. 7964(A)

Freshwater

Biological Association

RESEARCH ASSISTANT

Vacancy for Assistant Scientific Officer (ASO)—Scientific Officer (SO) to assist in work on the physiological ecology of planktonic algae and environmental factors of lakes, involving experimental and field studies. Two places at 'A' level (or equivalent) specialising in science, or a degree in biology, ecology, or environmental sciences.

Salary: ASO £2,188 (age 18), £2,665 (age 21) to £3,303 (max.); SO £2,839 to £4,415 (max.).

Further details and application form from: Secretary, Freshwater Biological Association, The Ferry House, Ambleside, Cumbria LA22 0EP. Telephone Windermere (096 62) 2468. Closing date: November 10, 1978. 7968(A)

UNIVERSITY OF
GLASGOWDEPARTMENTS OF
BIOCHEMISTRY AND
MEDICAL CARDIOLOGY

Applications are invited for the post of

RESEARCH ASSOCIATE

and

TECHNICIAN GRADE III

in a group investigating the structure and metabolism of cardiac muscle cells. For the research associate post a good Honours or Ph.D. degree in biochemistry plus experience in handling mammalian cells is desirable. Successful applicants will be working in the Department of Biochemistry.

Applications with curriculum vitae and naming two referees should be sent to Dr Jocelyn Dow, Departments of Biochemistry, University of Glasgow, Glasgow G12 8QQ. 8057(A)

WELSH NATIONAL
SCHOOL OF MEDICINE

(University of Wales)

DEPARTMENT OF MEDICAL

BIOCHEMISTRY

Applications are invited for a
POSTDOCTORAL
BIOCHEMIST

to work on the development of immunological assays of parathyroid hormone in man. The project (which is funded by the M.R.C. for a period of 3 years), involves the purification of parathyroid hormones from human and bovine tissue and the development of region-specific labelled antibody assays. Experience in protein purification and radioimmunoassay techniques is desirable.

Candidates should possess a Ph.D. in biochemistry. Salary on the scale for University Research and Analogous Staff Grade 1A; starting point up to £4,382 per annum.

Applications in the form of a brief curriculum vitae with the names and addresses of two referees should be sent to the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff CF4 4XN (quoting ref. No. C38/4/25). The Department may be visited by appointment with Dr J. S. Woodhead (Tel. Cardiff 755944, ext. 2803/2811). 8024(A)

UNIVERSITY OF GLASGOW

INSTITUTE OF VIROLOGY

POSTDOCTORAL

RESEARCH FELLOW

A vacancy exists for a Postdoctoral Fellow to work in a small team funded by the Cancer Research Campaign to study the relationship of herpes simplex virus to cervical carcinoma. It would be an advantage for candidate to have some experience in nucleic acid hybridisation techniques.

The position is tenable for three years, and the starting salary is in the range £3,883 to £5,129 p.a. depending on experience. U.S.S. Applicants should send a Curriculum vitae giving full details of qualifications and experience together with the names and addresses of two referees to:-

Dr N. M. Wylie,
Institute of Virology,
Church Street,
GLASGOW G11 5JR

as soon as possible.

7997(E)

ANTWERP UNIVERSITY
BELGIUM

CARDIOVASCULAR INSTITUTE

Applications are invited for a post of RESEARCH ASSISTANT, tenable for 1 year (starting 01.01.79) to investigate the ionic activities in heart cells.

Salary range: 546 840 BF to 793.379 BF

Candidates should have a good degree in biophysics, physiology, medicine or other related sciences with a particular interest in electrical properties of excitable membranes.

Applications giving details of education, experience and names and addresses of two referees should be sent by November 4, 1978 to Personnel Department, Universitaire Instelling Antwerpen, Universiteitsplein 1, 2610 Wilrijk, Belgium.

Further details can be obtained from Prof dr D. L. Brutsaert, Groenenborgerlaan 171, 2020 Antwerpen, tel. 031/30.59.80. 8034(A)

CHANGE OF ADDRESS

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**LANCHESTER
POLYTECHNIC
AT COVENTRY
DEPARTMENT OF
BIOLOGICAL SCIENCES
TECHNICIAN
(Animal Physiology)**

(£3,279 to £4,146 per annum)

For the servicing of laboratories, equipment and practical classes and other technical work mainly in animal physiology. Applicants should have qualifications at or above the level of N.C. or 'A' level including adequate physics and chemistry and sufficient relevant laboratory experience including some instrumentation.

**TECHNICIAN
(Biochemistry)**

(£3,279 to £4,146 per annum)

For the servicing of laboratories, equipment and practical classes and other technical work mainly in biochemistry and radiobiology. Applicants should have qualifications at or above the level of N.C. or 'A' level including adequate chemistry and have sufficient relevant laboratory experience including the handling of radioisotopes.

For application forms and job specifications for the above posts please apply in writing enclosing a foolscap stamped and addressed envelope to the Personnel Officer, Lanchester Polytechnic, Priory Street, Coventry CV1 5FB, returnable by October 30, 1978. 8059(A)

**BIOLOGIST/
PHYSIOLOGIST**

required by the Biology Section of the Medical Research Council Cyclotron Unit. The successful applicants will join a group investigating responses of normal tissues in animals which are relevant in the treatment of cancer. Studies will include effects of both neutrons and X-rays and certain adjuvant treatments used in radiotherapy. The post primarily will be concerned with damage to nervous tissue but may extend to other tissues. The applicant must have a First or Upper Second Class degree in an appropriate subject and at least 3 years' postgraduate research experience or a degree in medical or veterinary sciences. Relevant experience is desirable but not essential. The post is a three-year short-term appointment.

Salary, according to age and experience, in the range £4,815 to £6,180 (inclusive of pay supplements and London weighting), plus six weeks annual leave.

Please write, giving qualifications and curriculum vitae, to: Miss A. Pires, M.R.C. Cyclotron Unit, Hamcote Hospital, Duane Road, London W12 0HB. Tel: 01-743 4594, ext. 103. 8056(A)

NATURE CLASSIFIED

**will accept your
advertisement
up to 3.30 p.m.
Friday preceeding
Thursday's issue**

**UNIVERSITY OF
EDINBURGH
DEPARTMENT OF
BACTERIOLOGY
Postdoctoral Fellow/
Research Associate**

required to work on a Cancer Research Campaign sponsored project in tumour immunology. Experience in working with tumours or in the field of cellular immunology would be desirable. Salary on National Range 1A.

Send c.v. to Dr W. H. McBride, Department of Bacteriology, Edinburgh University Medical School, Teviot Place, Edinburgh EH8 9AG, Scotland. Please quote Reference 3058 7940(A)

**WEST MIDLANDS
REGIONAL HEALTH
AUTHORITY
BLOOD TRANSFUSION SERVICE
A vacancy exists for a
SCIENTIFIC OFFICER**

in the Laboratory in Birmingham. Applicants should be graduates in Biochemistry or Biological Sciences. Knowledge of Immunochemical Techniques and Protein Separation methods would be an advantage.

Salary scale, £3,486 to £4,275 per annum. Applications in writing to the Director, Blood Transfusion Service, Vincent Drive, Edgbaston, Birmingham B15 2SG. 8058(A)

TECHNICIAN

required at our Mill Hill Laboratories, London NW7, to join group working on experimental embryology and regeneration. Work will involve some animal maintenance (amphibia), histology, photography, laboratory maintenance and experimental work.

H.N.C./Degree. Laboratory experience an advantage. Salary range £3,261 to £4,902+L.W. Application form from Mrs Nelson, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2; Tel: 242 0200 ext. 305, quoting ref 13/79. Closing date for applications November 17, 1978. 8033(A)

OVERSEAS DEVELOPMENT

KNOW-HOW vital to developing countries

A 3-MAN TEAM consisting of:

- (i) TROPICAL AGRONOMIST
- (ii) SOIL PHYSICIST
- (iii) CROP PHYSIOLOGIST Botswana

is required for a Dryland Farming Research scheme—Phase III, situated at the Agricultural Research Station, Gaborone. The team will study edaphic and aerial factors affecting establishment of optimal stands of Sorghum and Maize for maximum yield over cycles of wet and dry seasons within the context of a new and modified farming system. The research is closely associated with projects evaluating the results under farmers' conditions and establishing a pilot scheme for their adoption. Applicants aged 30-55 years should be a graduate in science, agricultural or soil science with preferably previous overseas research experience.

Appointment 3 years. Salary in range £5,700 to £9,700 p.a. plus tax-free overseas allowance in range £1,360 to £3,375. (Ref. 331.)

These posts are wholly financed by the British Government under Britain's programme of aid to the developing countries. In addition to basic salary and overseas allowances other benefits normally include paid leave, free family passages, childrens' education allowances and holiday visits, free accommodation and medical attention. Applicants should be citizens of the United Kingdom.

For full details and application form please apply quoting reference stating post concerned, and giving details of age, qualifications and experience to:



Appointments Officer,
MINISTRY OF OVERSEAS DEVELOPMENT,
Room 301, Eland House,
Stag Place, London SW1E 5DH.

8018(A)

HELPING NATIONS HELP THEMSELVES

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RESEARCH OFFICER

The post is in the Laboratory for Allergens and is concerned with research on the standardisation of allergen extracts. The investigations involve both biochemical techniques and licensed animal work although the person appointed will be concerned more with the latter.

Applicants should be qualified to degree level or its equivalent (which included immunology), preferably with two years experience in relevant field work. Experience with lymphocyte cultures would be advantageous. Candidates should not have any known allergies.

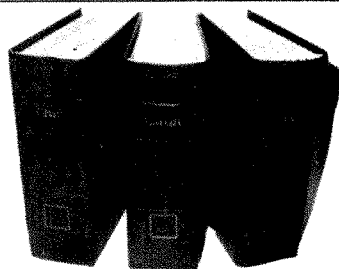
Salary, depending on qualifications and experience, on the scale £3,615 to £5,256 including London Weighting.

Application form and further details quoting Ref. No. 0168 from Personnel Officer, National Institute for Biological Standards and Control, Holly Hill, Hampstead NW3 6RB. Tel: 01-435 2232 ext 211. Returnable by November 10

NIBSC

National Institute for Biological Standards and Control
7990(A)

Nature Binders



Keep your copies of Nature protected in durable black PVC binders:

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 - * panel on spine for your own references
- Each binder is designed to take two volumes of Nature; three binders will serve for a year.

Prices (including postage): UK £3.00 each, three for £8.00
Rest of World US \$6.00, three for US \$16.00

Orders with correct remittance should be sent to:
Nature, Circulation Department, Macmillan Journals Ltd,
Brunel Road, Basingstoke, Hampshire RG21 2XS, England.

nature

CHANGE OF ADDRESS

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TELEPHONE : 01 831-6901

KINGSTON POLYTECHNIC SCHOOL OF CHEMICAL AND PHYSICAL SCIENCES SENIOR TECHNICIAN:

Applied Physics Laboratory

Duties include repair and maintenance of equipment and general maintenance of the laboratory; also providing assistance to students and staff in developing and running their experiments. Varied and interesting work to suit someone with drive and enthusiasm. T3/4 grade £4,017 to £4,917 inclusive.

TECHNICIAN

Postgraduate Research Laboratory

Duties include the maintenance and servicing of laboratory equipment plus general laboratory duties. It is expected that applicants will have at least 'A' level chemistry and be able to assist both research students and staff in various applications of their research. T2 grade £3,564 to £3,936 inclusive.

Further details and application forms from Assistant Registrar (Personnel), Kingston Polytechnic, Penrhyn Road, Kingston upon Thames KT1 2EE. Tel: 01-549 1366. 8051(A)

TECHNICIAN (Grade 5) with experience of histochemistry required in Department of Anatomy and Embryology. Knowledge of electron-microscopic techniques would be advantageous. This post is with an active research group and calls for adaptability and the capacity to give technical expertise and back-up to projects of varying duration. Salary in range £3,651 to £4,185 inc. of London Weighting (under review). Application form from Personnel Officer (Technical Staff FB38), University College London, Gower Street, WC1E 6BT. 7987(A)

LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE (University of London)

Keppel Street, WC1E 7HT

DEPARTMENT OF MEDICAL MICROBIOLOGY

A MEDICAL LABORATORY SCIENTIFIC OFFICER/ SENIOR SCIENTIFIC OFFICER

is required to work up to two years on the viruses of African haemorrhagic fevers. Candidates must be graduates or hold HNC/HND or an equivalent qualification and have had experience in electron microscopy.

Inclusive salary, depending on experience, rises to a maximum of £4,284 (Scientific Officer) and £6,123 (Senior Scientific Officer). Pensionable employment.

Applications, consisting of full career and education details and naming two referees, should be sent to Secretary (A1) at the School. 8044(A)

Junior Laboratory Scientific Officer required in Department of Microbiology. Some experience of cell culture and virology preferable. Salary according to age and qualifications. Apply in writing, stating age and giving details of qualifications and experience, to the Secretary, Guy's Hospital Medical School, London Bridge SE1 9RT, quoting Ref. M.C.4. 7890(A)

INVERTEBRATE PALEONTOLOGIST UNIVERSITY OF CINCINNATI

A tenure-track faculty position in invertebrate paleontology will be available at the University of Cincinnati as of September 1, 1979. We are seeking a person with demonstrated research experience in invertebrate paleontology. The Ph.D. is required. Candidates should possess a thorough knowledge of systematics and the ability to apply this knowledge to a wide range of problems such as functional morphology, paleoecology, biostratigraphy, or phylogeny. Teaching duties will be at the undergraduate and graduate levels, and the person will supervise both M.S. and Ph.D. theses. The person will also be expected to maintain an active research program and cooperate in the departmental program in paleontology. Rank and salary are negotiable. Deadline for receipt of applications is January 31, 1979.

Applications should include: (1) a brief statement of research interest and goals; (2) curriculum vitae and experience; (3) complete bibliography; and (4) names of three references. Applications should be submitted to:

Dr David L. Meyer,
Department of Geology,
University of Cincinnati
Cincinnati, Ohio 45221.

The University of Cincinnati is an Equal Opportunity/Affirmative Action Employer 8021(A)

university of wales university college of swansea

Lecturer

Applications are invited from candidates with a good honours degree for a vacancy of **LECTURER** in the Department of Botany and Microbiology. Preference may be given to applicants with interests in Environmental Botany.

The appointment, which is tenable from January 1, 1979, will be made on the Lecturers' Scale up to £4,882 per annum, together with U.S.S./U.S.D.P.S. benefits.

Further particulars and application forms (2 copies) may be obtained from the Personnel Officer, University College of Swansea, Singleton Park, Swansea SA2 8PP, to whom they should be returned by FRIDAY, NOVEMBER 10, 1978. 8065(A)

UNIVERSITY OF LEICESTER

DEPARTMENT OF PHYSIOLOGY Applications are invited for the post of LECTURER

in the Department of Physiology. A interest in teaching medical students is desirable. There are excellent research facilities.

Initial salary, dependent on qualifications and experience, within the scale £3,883 to £7,754 with superannuation benefits.

Further particulars from the Registrar, to whom applications should be sent by November 3, 1978, quoting Reference NLP. 8050(A)

APPOINTMENTS VACANT

THE ROWETT
RESEARCH INSTITUTE
Buckburn, Aberdeen AB2 9SB
APPLIED NUTRITION
DEPARTMENT

Applications are invited to fill a vacancy in the Institute's Applied Nutrition Department. The successful candidate will work on the effects of nutrition and of management on reproduction in the sow, and particularly on ways in which the frequency of breeding can be increased. The work would entail studies of the artificial rearing of baby pigs.

Candidates should have a first or upper second class honours degree in the biological sciences or agriculture or equivalent qualification.

The appointment will be as a

SCIENTIFIC OFFICER

(£3,294 to £4,415 p.a.)

or a

HIGHER

SCIENTIFIC OFFICER

(£4,101 to £5,448 p.a.)

grade and starting salary depends on experience. At least two years' relevant postgraduate experience is required for H.S.O. grading.

There is a non-contributory pension scheme.

Further details of the post may be obtained from the Secretary of the Institute, to whom applications should be submitted together with the names of two people to whom reference can be made. Closing date, November 3, 1978. 8068(A)

ELSEVIER INVITES
APPLICATIONS FOR AN
ACQUISITION EDITOR
IN AMSTERDAM

Elsevier Scientific Publishing Company, Amsterdam, The Netherlands, invites applications for the position of Acquisition Editor in the department of Engineering and Technology. After a training period, the successful candidate will, together with his/her colleagues in the department, further develop the Elsevier publication programmes in this field.

Candidates are expected to hold a university degree in an appropriate subject, which could be civil, chemical, mechanical, electrical engineering, or geology or materials science. Experience as an acquisition editor in these fields would be an advantage but is not essential. He or she should be able to communicate clearly and easily, be willing to move to Amsterdam, be under 35, and be keen to work outside a narrow specialisation.

Applications with full curriculum vitae, passport photograph, and an indication of present and expected salary should be sent to the Head, Personnel Department, Elsevier Scientific Publishing Company, P.O. Box 2400, Amsterdam, The Netherlands 7976(A)

PRE AND POSTDOCTORAL
POSITIONS

are available for studies on the protein synthesising system with special emphasis on ribosomes—topography, biogenesis and function—under various conditions of growth and development. Teaching opportunities available. Reply with résumé to:

Professor Bruce H. Sells,
Laboratories of Molecular Biology,
Faculty of Medicine,
Health Sciences Complex,
Memorial University,
of Newfoundland. 8003(A)

Accelerator Physicists for SNS Rutherford Laboratory

The Rutherford Laboratory of the Science Research Council has recently undertaken to design and construct a major new experimental facility known as the Spallation Neutron Source (SNS). This will consist of a fast cycling 800 MeV proton synchrotron delivering 2.5×10^{13} protons per pulse at 50Hz onto a heavy target to produce an intense source of neutrons. When completed the SNS will be a world class facility serving a wide community of research workers in the study of condensed matter by neutron scattering techniques.

The Laboratory is now seeking a number of young physicists to join the design team on this advanced and challenging project. Vacancies are available in a number of areas of the work which includes such topics as accelerator machine theory, magnet design, vacuum technology, high power rf systems, electronics, control systems and target design.

Suitable candidates will have graduated within the last few years and will be qualified either to carry out original design and development work, or to provide experimental or theoretical support within a project group.

Appointment will be at either the Scientific Officer or the Higher Scientific Officer level, depending on age and experience. Salary will be in the range:

Scientific Officer — £3037 - £4724

Higher Scientific Officer — £4388 - £5829

There is a contributory pension scheme, generous leave and sick leave allowances.

The Rutherford Laboratory is situated on the Berkshire Downs, about 18 miles south of Oxford. It has its own restaurant and recreational facilities. Shops, banking facilities, hostels and further sports and recreational clubs are available on the adjoining AERE Harwell site. Daily travel is available from most of the neighbouring towns and villages direct to the Laboratory.

Application forms are available from:

RUTH JEANS of the Personnel Group.

Please quote reference No. VN 747.

Closing date for applications

31st October 1978

RUTHERFORD
Science Research Council
Rutherford Laboratory, Chilton, Didcot,
Oxfordshire OX11 0QX. Tel Abingdon 21900

8069(A)

UNIVERSITY OF ALBERTA,
EDMONTON, CANADA. Faculty
position vacant in Department of
Medical Bacteriology. Rank and salary
negotiable. Medical degree and inter-
ests in antibiotics or mechanisms
of pathogenicity desirable. Applications
with curriculum vitae to the Chairman,
Professor F. L. Jackson, University of
Alberta, Department of Medical Bacteri-
ology, Edmonton, Alberta, Canada
T6G 2H7. 8055(A)

EDITORIAL ASSISTANT. General
Practitioner, the weekly medical news-
paper, needs an assistant to the science
editor. He/she will be a recent gradu-
ate in one of the biological sciences.
Duties will include commissioning and
editing clinical articles. Some writing
involved, but experience not essential.
Send full details to Jenny Bryan,
Science Editor, General Practitioner,
Haymarket Publishing, 54-62, Regent
Street, London W1A 4YJ. 8025(A)

Qualified Laboratory Scientific Officer
required in Physiology Department, to
assist with teaching laboratory duties
and research. Salary not less than
£3,261 on scale rising to £4,680, plus
£354 London Weighting. Apply in
writing to the Secretary, Guy's Hos-
pital Medical School, London Bridge,
SE1 9RT, quoting Ref. P.Y.1.
7994(A)

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CHAIRPERSON
DEPARTMENT OF PHARMACOLOGY
UNIVERSITY
OF PENNSYLVANIA

Application is invited from highly qualified individuals to head the Department of Pharmacology of The University of Pennsylvania School of Medicine.

Reply to Pharmacology Search Committee, Dean's Office, School of Medicine, University of Pennsylvania Philadelphia, PA. 19104. An equal opportunity employer.

8062(A)

OPEN UNIVERSITY
PRODUCTIONS
SCIENCE

Applicants are invited for one post at Production Assistant level. Candidates should have a good Honours Degree in Geology and at least two years post-University experience. The successful applicant will work on all aspects of the production of television and radio programmes for the Open University and will be expected to work on a wide range of subject disciplines. An informed interest in Science and a desire to work in the fields of communication of Science by Television and Radio is essential.

Open University Productions is based at Alexandra Palace but is expected to move to Milton Keynes in 1981.

Salary: £5,185 p.a. (may be higher if qualifications are exceptional) x £195 to £8,755 p.a. plus £410 p.a. for unsocial hours. Relocation expenses considered.

Telephone or write immediately for an application form, enclosing addressed envelope and quoting reference number 78.G.2580.NA. to Appointments Department BBC, London W1A 1AA. Telephone: 01-580 4468 ext. 4819.



8053(A)

UNIVERSITY OF
EDINBURGH
DEPARTMENT OF
PHARMACOLOGY
POSTDOCTORAL
RESEARCH FELLOW/
CHEMIST

Applications are invited for a Research Fellowship to work on the chemical synthesis of prostaglandin analogues with Drs R. L. Jones and N. H. Wilson. The appointment is for three years and carries a starting salary within the range £3,660 to £4,360 per annum depending on age and experience. (This salary scale (IA) is under review and carries F.S.S.U./U.S.S. benefits.) Comprehensive facilities for synthetic chemistry are available in the department.

Applications, with curriculum vitae, and the names of referees, should be sent as soon as possible to Dr N. H. Wilson, Department of Pharmacology, University of Edinburgh, 1 George Square, Edinburgh EH8 9JZ. Please quote Reference 5046 7993(A)

QUEEN CHARLOTTE'S
MATERNITY HOSPITAL
Goldhawk Road, London W6
BASIC GRADE OR SENIOR
SCIENTIFIC OFFICER
(Cytogenetics)

Applications are invited from Science Graduates with honours degrees for the above posts. Preference will be given to candidates with experience of diagnostic work in human cytogenetics. Whitley Council terms and conditions of service. The diagnostic laboratory, which is fully equipped, may be visited by appointment. Intending applicants are advised to contact Dr D. Hughes (Tel. 01-748 4666, Ext. 223) in the first instance.

Application forms obtainable from and returnable to the Administrative Assistant (Ext. 204) 8075(A)

UNIVERSITY OF OXFORD
INORGANIC CHEMISTRY
LABORATORY

An S.R.C. POSTDOCTORAL APPOINTMENT in Organo-transition Metal Chemistry is available from January 1, 1979. The work concerns the synthesis and study of organometallic compounds. Experience with metal vapours or photochemistry would be advantageous.

For details please contact Dr M. L. H. Green, Inorganic Chemistry Laboratory, South Parks Road, Oxford OX1 3QR 8071(A)

APPOINTMENTS WANTED

Ph.D 18 years' biochemical research seeks part-time employment, London area, eg. free-lance editorial, but anything considered. Write to Box 8074(A) Nature, Macmillan Journals, 3 Dyers Buildings, London EC1N 2NR. 8074(A)

APPOINTMENTS VACANT

OXFORD UNIVERSITY
DEPARTMENT OF
PHARMACOLOGY

A vacancy has occurred in the above department for a

GRADE 4 TECHNICIAN

in a unit interested in radioenzymatic and radioimmunoassays. Some biochemical or immunological experience would be desirable. Research is directed towards brain mechanisms. Qualifications needed are some 'O' levels and 2 'A' levels in appropriate subjects. This appointment is initially for 3 years on an M.R.C. grant.

Salary and hours as per University scales and conditions.

Apply in writing to The Administrator, Department of Pharmacology, South Parks Road, Oxford OX1 3QT 8063(A)

INSTITUTE OF
DERMATOLOGY

(University of London)

A JUNIOR TECHNICIAN

Is required to work in the Professional Unit. Applicants will be expected to have 'A' levels in biology and chemistry or equivalent qualifications and the successful applicant will assist in studies to determine levels of mediators in human skin and other tissue.

Applications giving full details of age, qualifications and experience together with the name of 2 referees should be sent to Mrs V. M. Plummer, Professional Unit, Institute of Dermatology, Homerton Grove, London E9 6BX. 8072(A)

UNIVERSITY OF BRISTOL
DEPARTMENT OF PHYSICS
TECHNICIAN GRADE 5

Applications are invited for the above post involving operation and maintenance of a Cambridge S4 Scanning Electron Microscope with associated optical, electronic and photographic equipment used chiefly in cathodoluminescence experiments. Other duties include operation and maintenance of X-ray apparatus and of equipment for cutting and polishing crystals. Plenty of scope exists for initiative in assisting with new developments. Previous experience in above-mentioned specialised work not essential, but experience in general laboratory practice including vacuum technique and some electronics is desirable.

Salary within the range £3,186 to £3,720 per annum (scale under review), and the position is funded for two years.

Applications, stating age, experience and qualifications should be sent as soon as possible to Dr A. R. Lang, H. H. Wills Physics Laboratory, Tyndall Avenue, Bristol BS8 1TL. 8073(A)

MISCELLANEOUS

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UNIVERSITY OF EDINBURGH

TAIT CHAIR OF MATHEMATICAL PHYSICS

Applications are invited for the Tait Chair of Mathematical Physics which will fall vacant on the retirement of Professor N. Kemmer, F.R.S. on September 30, 1979. Applications from theorists in any branch of Physics will be considered.

Salary within the professional range, with U.S.S./F.S.S.U.

Further particulars may be obtained from

The Secretary to the University
University of Edinburgh
Old College
South Bridge
Edinburgh EH9 9YL

with whom applications (12 copies), giving the names of three referees, should be lodged by December 15 if possible. (Overseas candidates need submit only one copy of their application.) Please quote reference 13/78. 8013(A)

RHODES UNIVERSITY GRAHAMSTOWN SOUTH AFRICA

Applications are invited for the post of

PROFESSOR OF ENTOMOLOGY

with effect from January 1, 1979 or as soon as possible thereafter.

The initial salary will be determined according to qualifications and experience within the salary scale R10,800 by R450 to R12,600 by R600 to R13,800 per annum. In addition a supplement of 15% per cent on the above scale and a vacation savings bonus are payable. (Note: £1 sterling equals approximately R1.73). The successful applicant will become a member of the University's pension and medical aid schemes.

Preference will be given to candidates with teaching and research interests in the field of insect ecology or physiology.

Further particulars and application forms may be obtained from the Registrar (Personnel Section), Rhodes University, Grahamstown, South Africa, to whom completed applications together with a recent photograph should be sent by November 4, 1978. 7978(A)

AWARDS

AUSTRALIAN INSTITUTE OF MARINE SCIENCE Postdoctoral Awards

The Australian Institute of Marine Science is a Statutory Authority established in 1972. Its functions are to carry out research in marine science, to assist other persons and institutions engaged in research in this field and to disseminate information relating to marine science.

The Institute is located in modern facilities at Cape Ferguson 60 kilometers from Townsville.

A number of Postdoctoral Awards are granted each year by the Council of the Institute for research by young scientists of exceptional promise and proven capacity for original work in the area of marine science.

The awards are tenable at the Institute for periods up to two years. Tenure of an award is expected to commence within nine months of the award being made.

In general, awards are restricted to applicants who are not more than 30 years of age on the date when applications close. Applications may also be considered from candidates for the Ph.D. degree if a thesis has been submitted for examination.

Stipend

\$10,059 to \$14,064 per annum.

Allowance

A district allowance of \$340 per annum for married awardees and \$180 for single awardees is payable.

Applications

Persons interested in applying for an award should submit an application and the names and addresses of three professional referees to the Secretary, Australian Institute of Marine Science, P.M.B. No. 3, M.S.O. Townsville, Queensland 4810, Australia.

Each application should be accompanied by a curriculum vitae and should detail a research proposal with scientific justification, procedures and objectives. Details of necessary laboratory and/or field support should be documented.

Applications should be submitted before the 15 December, 1978. 8068(A)

FELLOWSHIPS

UNIVERSITY OF STRATHCLYDE

Applications are invited from graduates in engineering, physics or other relevant disciplines for

TWO RESEARCH FELLOWSHIPS

In the BIOENGINEERING UNIT to work in the Unit's programme of investigation of biomechanics of human movement. Preference will be given to candidates with a research degree and with experience in the measurement of human biomechanics.

One Research Fellowship, financed by the Scottish Home and Health Department, is involved with the investigation of the reproducibility of prosthetic limb alignment and the changes in structural loading produced by controlled alterations in alignment.

The other Research Fellowship, financed by the Medical Research Council, is concerned with the investigation of the loading of both hips and both knee joints during normal activity for patients with joint disease at one or more of these sites. Patients will be tested before and at intervals after joint replacement.

Both posts are for a period of three years and are on Range 1A of the national salary structure for research and analogous staff. The S.H.H.D. Fellowship will be on the scale £4,382 by £249 to £4,631 by £251 to £4,882 and the M.R.C. Fellowship on the scale £4,631 by £251 to £4,882 by £247 to £5,129. Placing on respective scales according to qualifications and experience. Superannuation benefit.

Written applications with the names and addresses of two referees (quoting R38/78) should be forwarded to Professor J. P. Paul, Bioengineering Unit, Wolfson Centre, University of Strathclyde, 106 Rottenrow, Glasgow G4 0NW (Tel. 041-552-4400, ext. 3030), from whom further details can be obtained. Please state in which post interested. 8029(E)

UNIVERSITY OF LIVERPOOL DEPARTMENT OF IMMUNOLOGY

Applications are invited for the post of postdoctoral research fellow in the Department of Immunology to work with Dr P. M. Johnson on the interaction of immunological responses to oncofetal antigens with tumour promotion or inhibition in syngeneic experimental animal systems. Previous experience in cell biology or tumour immunology would be advantageous. Initial salary will be in the range £3,883 to £4,631 per annum.

Applications together with the names of two referees should be received not later than November 3, 1978, by the Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further details may be obtained. Quote Ref: RV/961/N 8008(E)

AGRICULTURAL RESEARCH COUNCIL ANIMAL BREEDING RESEARCH ORGANISATION

Applications are invited for a vacancy in the Department of Growth and Efficiency for work on the genetics of growth and productive efficiency of livestock.

Candidates should have a first or upper second class Honours Degree or the equivalent in an appropriate subject and at least two years' post-qualifying experience in mathematics, statistics, genetics or animal science. They should have the ability to conduct and direct large-scale experiments on cattle fed a controlled complete diet. An ability to analyse data and use extensive computing facilities is essential.

Appointment as Higher Scientific Officer (£4,101 to £5,448), starting salary depending on experience. Non-contributory superannuation.

Further information and application forms are available from the Secretary, ARC Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ. Closing date November 11, 1978. 8012(A)

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FELLOWSHIPS

UNIVERSITY COLLEGE CORK
FISH PATHOLOGY
 Post-Doctoral Fellowship

The National Board for Science and Technology has provided a grant for a study of kidney diseases of cultured salmon and trout.

The appointment will be for one year in the first instance renewable for a further two years.

STARTING SALARY: £3,762 per annum.

Applications with curriculum vitae and the names of two referees should be sent to:

M. F. Mulcahy, Ph.D.,
 Department of Zoology,
 University College, Cork
 (Ireland)

CLOSING DATE: Friday, 3 November, 1978

8010(E)

GIRTON COLLEGE
 CAMBRIDGE

Applications are invited for a scientific research fellowship open to graduates of any university and tenable for three years from October 1, 1979. The applications should be in the field of Mathematics, Natural Sciences, Geography and allied subjects.

The fellowship is to the value of £2,475 per annum to a candidate with a Ph.D. and £2,255 per annum to a candidate who has not completed a Ph.D. The fellowship is pensionable under U.S.S.

Particulars are available from the Secretary to the Council, Girton College, Cambridge CB3 0JG to whom applications should be sent by November 15, 1978. 7913(E)

UNIVERSITY OF
 BIRMINGHAM
 DEPARTMENT OF
 MICROBIOLOGY
RESEARCH FELLOWSHIP

A postdoctoral research fellow required for studies on the pathogenicity of influenza virus supported by the Medical Research Council. Virological training preferred but not essential. The post is for a Research Fellow 1A in the range £3,883 to £6,555, (plus superannuation), beginning January 1, 1979 for two years and nine months. Maximum starting salary will be £4,862.

Applications should be sent to the Assistant Registrar, Science and Engineering, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, by Friday, November 3, 1978, and further details can be obtained from Professor H. Smith. Please quote ref: NM3. 8004(E)

**POSTDOCTORAL
 RESEARCH
 FELLOWSHIPS IN
 BIOCHEMISTRY**

Applications are invited for two, two-year postdoctoral research positions in the Division of Molecular and Cellular Biology at the National Jewish Hospital and Research Center. The positions involve the study of an *in vitro* RNA processing system (see *Proc. Nat. Acad. Sci. USA* 75, p. 3045, 1978) and the structure and function of ribosomal RNA (see *Biochemistry* 16, p. 5321, 1977). Experience with nucleic acid chemistry and/or the analysis of complex enzyme kinetics is desirable. The beginning salaries are \$11,000 U.S. per year, with some allowance for experience and dependents. Application, with curriculum vitae and names of three referees, should be sent to Dr Norman Pace, N.J.H.R.C., 3800 E. Colfax Avenue, Denver, Colorado 80206 U.S.A. An Affirmative Action/Equal Opportunity Employer 8000(E)

CONFERENCES

CONFERENCE

Third International Conference
 on the Chemistry and
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 1/3 Grosvenor Place,
 London SW1X 7DL. 8034(C)

ANNOUNCEMENTS

The Royal Zoological Society of N.S.W. announces publication of the proceedings of a symposium on **MONOTREME BIOLOGY** held in May 1978. The paperback volume contains both review and research papers on topics of monotreme biology ranging from the fossil record to molecular physiology. Cost is \$3.00 (AUST) payable to The Royal Zoological Society of N.S.W., P.O. Box 20, Mosman N.S.W. 2088, AUSTRALIA 7980(G)

POSITIONS WANTED

AGRONOMIST (B.A., M.A.) (Cantab.)
SPECIALIST RESEARCH/ADVISORY AGRONOMIST IN TEA seeks appointment with any Research or Advisory Organisation, or Tea Company on Consultancy or Short Term Contract Basis. In my 50's and offer over 22 years' Research and Advisory experience in Ceylon, East and West Africa, Madagascar and Morocco. BILINGUAL, Fluent French. Also 24 years SUGAR CANE Research, NATAL. 7977(B)

STUDENTSHIPS

UNIVERSITY OF
 LIVERPOOL
 S.R.C. C.A.S.E.
**STUDENTSHIP IN
 MATERIALS SCIENCE**

Applications are invited for a studentship on a project concerned with the structure of non-metallic materials injected with light gas ions. The research will be undertaken in collaboration with the Culham Laboratory, who will make a payment to the student in addition to the S.R.C. studentship. The normal S.R.C. requirements will apply. Applications should be submitted as soon as possible to The Registrar, The University, P.O. Box 147, Liverpool L69 3BX. Quote Ref. RV/959/N. 8007(F)

ASSISTANTSHIPS

UNIVERSITY OF BRISTOL
 Applications are invited for a
POSTDOCTORAL

RESEARCH ASSISTANTSHIP in the Department of Zoology, work on the mechanics of flight, birds and bats, using wake sampling techniques. Candidates may be formally qualified either in zoology, or aeronautical engineering or a related field. They must be able to work with tame animals, have some practical knowledge of electronics and photography, and be proficient at computer programming.

Starting salary up to £4,382 p.a. (scale under review).

Further details from Dr C. J. Pennicock, Department of Zoology, University of Bristol, Woodland Road, Bristol BS8 1UG. 7983(F)

**POSITION IN
 INVERTEBRATE ZOOLOGY
 DEPARTMENT OF
 ZOOLOGY
 UNIVERSITY OF
 CALIFORNIA, BERKELEY**

The Department of Zoology, University of California, Berkeley, anticipates making an appointment at the assistant professor level beginning Fall, 1979 pending final budgetary approval. I successful applicant will be expected to participate in teaching a two quarter upper division course in invertebrate zoology and to offer an advanced course in his/her specialty. Preference will be given to individuals with teaching and research specialization in invertebrate morphology, physiology, endocrinology (excluding neurophysiology and behaviour). Applicants should send a curriculum vitae, copy of publications, a detailed statement of teaching and research objectives and names and addresses of at least three references to: Chairman, Invertebrate Zoology Search Committee, Department of Zoology, University of California, Berkeley, CA., 94720. Applications should be received January 1, 1979.

The University of California is an Affirmative Action Employer. 8015(P)

SYMPOSIA

**SIXTH SUMMER PROGRAM
 in
 METHODS OF
 IMMUNOLOGIC RESEARCH
 AND DIAGNOSIS**

The sixth biennial program on current methods of immunologic research and diagnosis will be offered by The Center for Immunology of State University of New York at Buffalo, June 4-22, 1979. It will consist primarily of a combination of lecture and elective practical laboratory courses to allow the participant to become acquainted with the techniques by personally carrying them out, the bench complemented by demonstrations, lectures and discussions. The entire program is designed to provide the participant with a survey of presently available methodology and insight into the underlying immunologic principles.

Further information may be obtained from James F. Mohn, M.D., Director, The Center for Immunology, State University of New York at Buffalo, 210 Sherman Hall, Buffalo, New York 14214. The deadline for applications is March 31, 1979. 8031(M)



UNIVERSITY OF DUBLIN
 Trinity College

**POSTDOCTORAL
 FELLOWSHIP/RESEARCH
 ASSISTANTSHIP**
 Department of Biochemistry
 Trinity College,
 Dublin 2.

A Position is available for a Postdoctoral Fellowship/Research Assistantship to participate in a Research Project designed to affinity-label and purify the substrate-binding components of a neutral amino acid transport system from plasma membranes of a protozoan.

The position is funded by the Medical Research Council (Ireland) and is for three years at a starting salary of £2,433 (Tax Free).

Candidates will possess an honours degree in Biochemistry or Chemistry and have interests in protein chemistry and cell biology. Enquiries or applications with a brief curriculum vitae to Dr H. P. Voorhees, Department of Biochemistry, Trinity College, Dublin. 8009(E)

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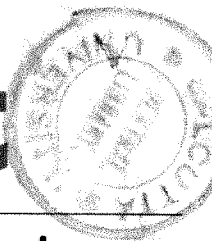
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Our first translation kit saved you the difficulty of preparing your own lysate for cell-free translation studies, gave you a system free of endogenous mRNA and high in protein synthesis activity, and assured you of components tested for compatibility and reproducible results. All told, it was an excellent excuse to stop cultivating rabbits.

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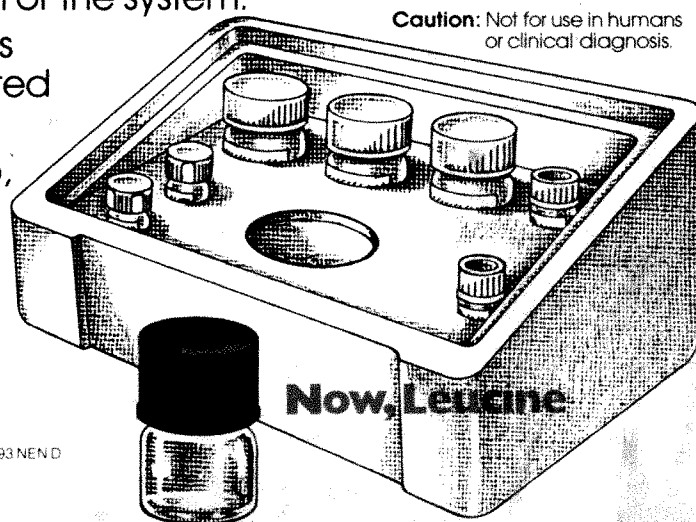
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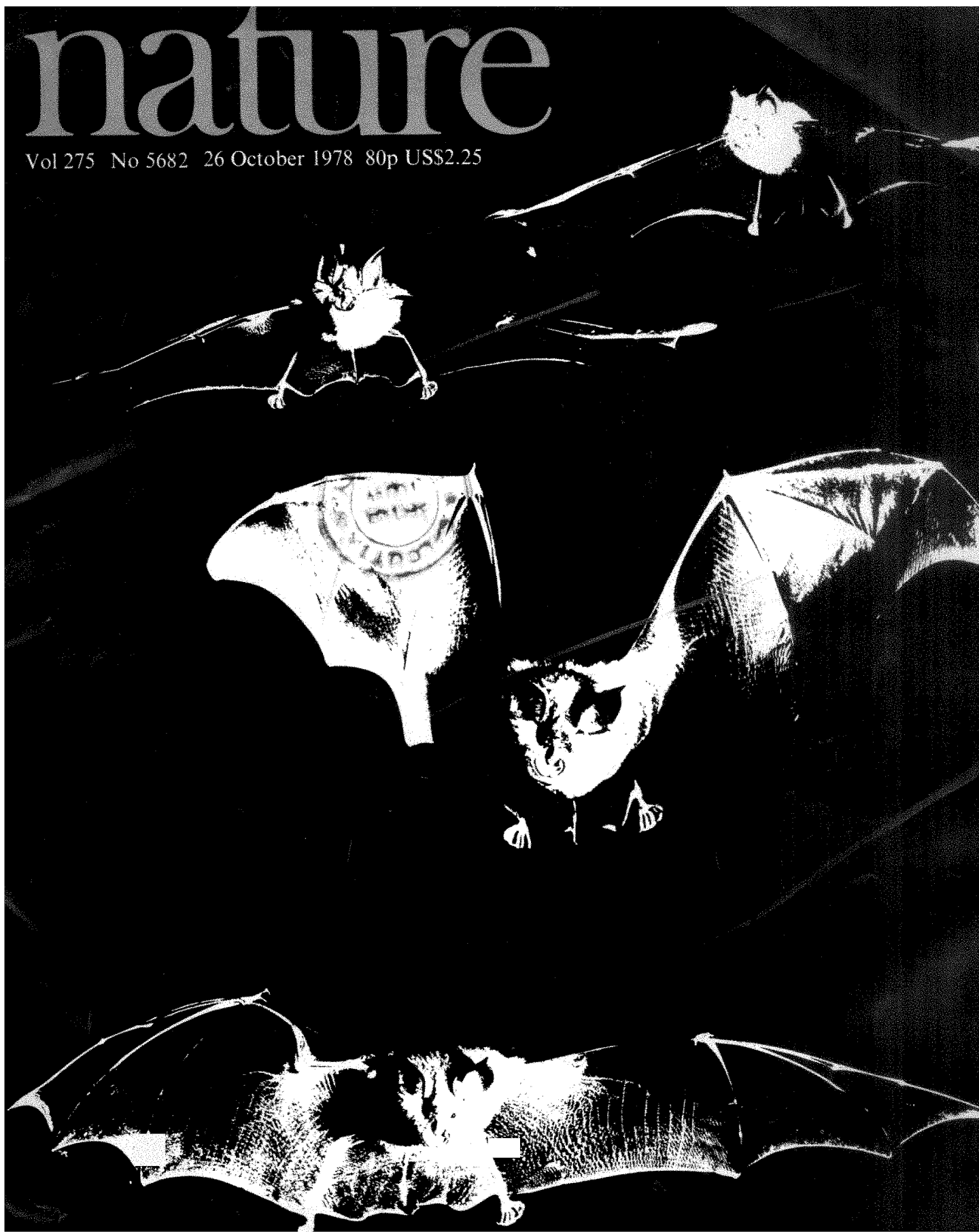
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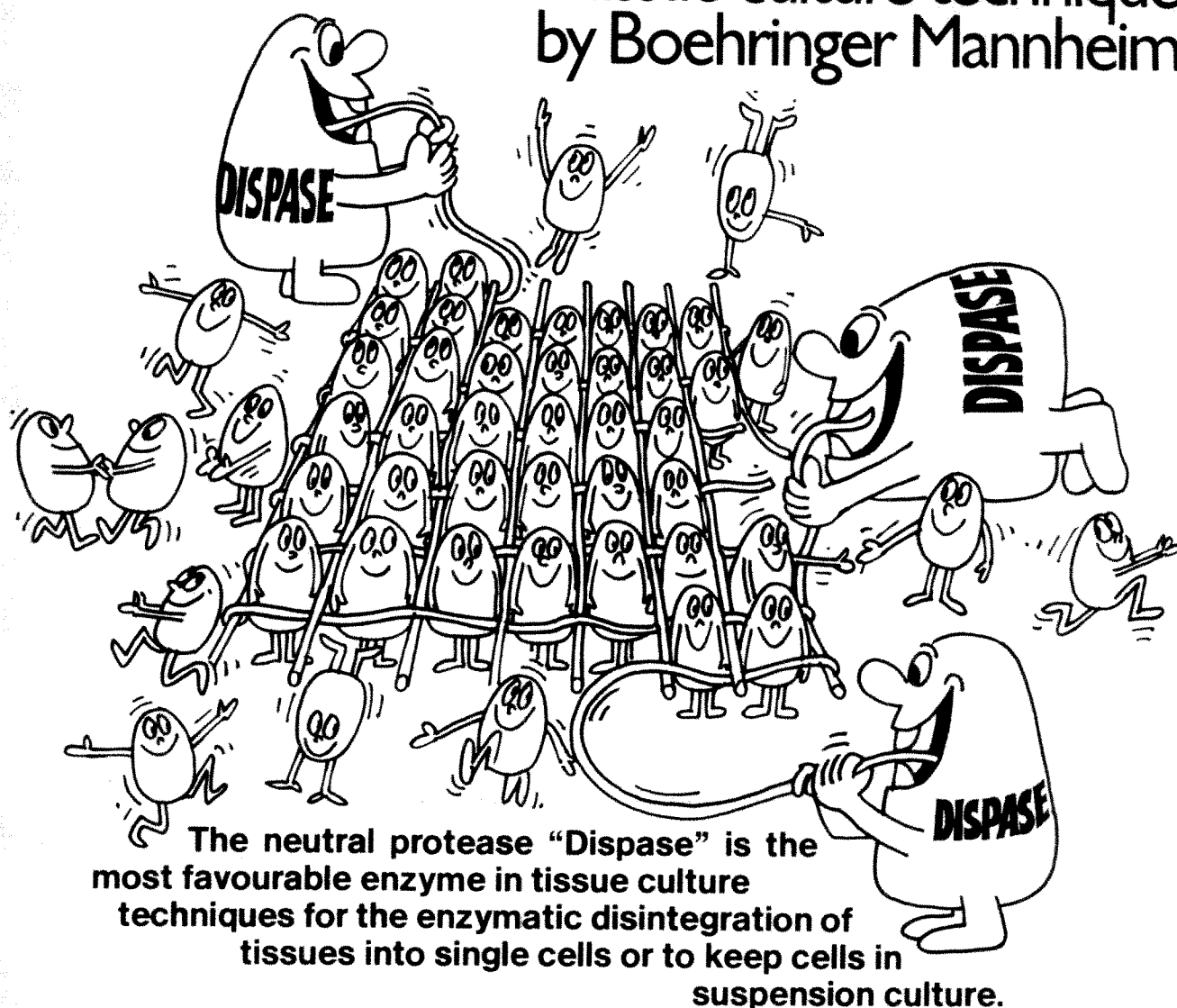
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Cover picture

Bats are able to use their echolocation
system more effectively in avoiding
moving objects than stationary ones.
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[Sdeuard Bissierot and John Topham]

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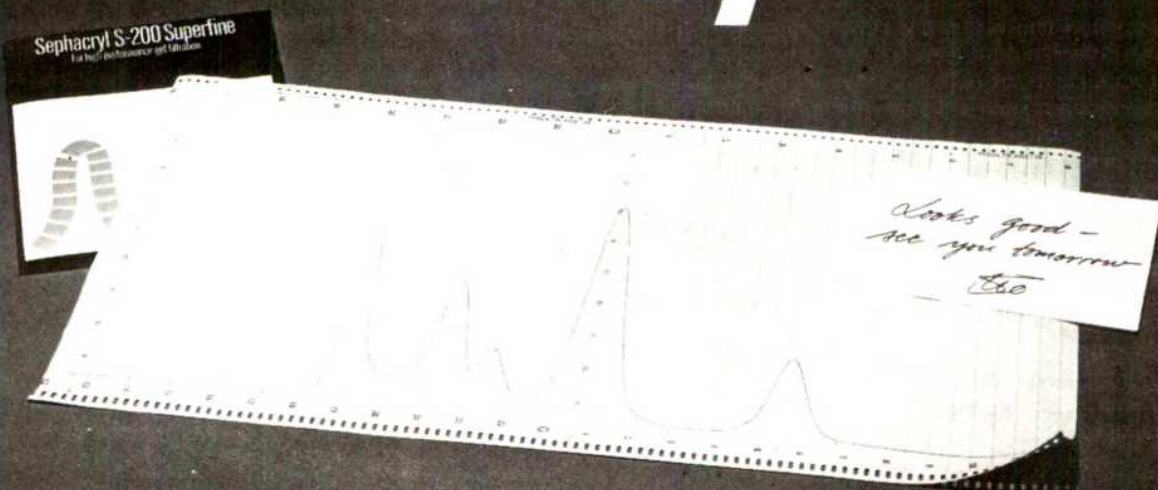
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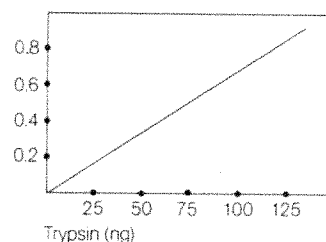
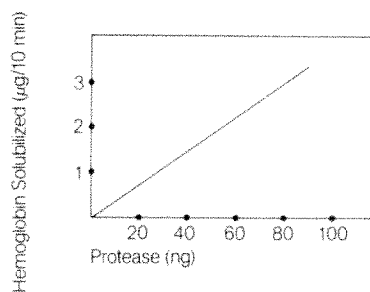
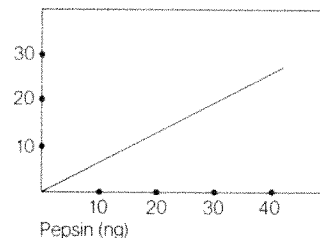
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nature

26 October 1978

Is proliferation unstoppable?

IN a mere eighteen months' time the Non-Proliferation Treaty (NPT) will come up for its second review conference. The treaty, which came into force in 1970, is intended to prevent non-nuclear-weapon states building their own nuclear devices or receiving them from any other source; in return nuclear-weapon states promise not to transfer nuclear explosives, nor to help others build their own. A majority of all nations adhere to the treaty, but there are a number of potentially nuclear countries who have kept out. West Germany, Japan, Sweden and Switzerland are in, but Argentina, Brazil, France, India, Israel, Pakistan, People's Republic of China and South Africa are not. This latter group contains, of course, three nuclear states, two of which are widely regarded as virtually nuclear and others which could harbour nuclear-weapons ambitions. So the NPT could hardly be said to be an unqualified success.

The first review of the treaty came up in 1975 and was not a happy occasion. The superpowers wanted to emerge unscathed from the conference and were largely successful. The price for this, however, was growing dissatisfaction amongst non-nuclear-weapons states, notably Mexico and Yugoslavia, at the way their self-denial had met with no real promises of restraint on vertical proliferation. In the event no-one withdrew from the treaty, but on the other hand no outsider proceeded to sign.

The 1980 conference could be much more of a cross-roads. Certainly the superpowers will bring a new Strategic Arms Limitation Treaty, and, most likely, a short-duration Comprehensive Test Ban as evidence of self-restraint, though both of these will be heavily criticised. But the real issues will probably be elsewhere. In the past few years, security of future energy supply has tended to supplant other world concerns, including nuclear proliferation. So uranium enrichment, reprocessing and breeder reactors are likely to be topics of importance at the 1980 conference, and the central debate could well focus on the right of nations to have access to these facilities. The Stockholm International Peace Research Institute, SIPRI, has recently held a conference to look at the control of fissionable materials in non-military applications, with a view to stimulating thought on these matters during the run-up to 1980. There are clearly many areas of great concern. Traditional methods of uranium enrichment could be challenged soon by jet-nozzle and vortex-tube techniques in West Germany and South Africa, as well as by laser separation methods. Reprocessing facilities capable of supplying plutonium are being developed in several countries with an eye on export and with the apparent justification of the Non-Proliferation Treaty itself, whose Article IV urges states to cooperate in the further development of peaceful uses of nuclear energy. The technological momentum of breeder research and development is making that device look like the logical next step for some nations, and it has the appeal of enormous savings on resources. Enrichment, reprocessing and breeding all look attractive in the absolute, but all open new doors to the more widespread circulation of weapons-grade materials.

What, the SIPRI meeting asked, is to be set against these proliferative trends? The answer seems to be that no one measure, whether technological, political or institutional

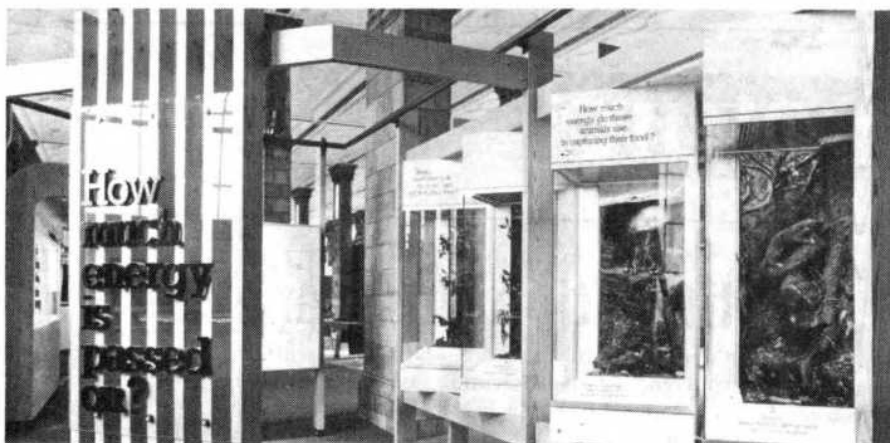
can in itself stem the tide for long; the best that can be hoped for is that by applying as many restraints as possible and in as many ways as possible, non-nuclear countries considering the acquisition of nuclear weapons delay long enough for new attitudes to have emerged, both towards proliferation and towards the role of nuclear energy in national energy strategies. Amongst subjects discussed were the possibility of the International Nuclear Fuel Cycle Evaluation (INFCE) coming up with something valuable; the chances that an Advanced Converter Reactor development in the United States would lead to efficiencies which made a breeder reactor seem unnecessary, at least for the present, possible use of denatured fuel; moratoria on various enrichment technologies and problems posed by fusion-fission hybrids, particularly using inertial confinement techniques. And yet the question ultimately is not technological but political and institutional. Energy independence has become a target, and with many years of almost unbridled enthusiasm for the atom not only in national but also international agencies there is much to be done before new attitudes emerge.

The most overtly political and apparently progressive move so far—President Carter's 1978 Non-Proliferation Act—has not been well-received everywhere. The Act puts strong controls on trading of nuclear materials and equipment originating in the United States, but in exchange tries to assure adequate safeguarded supplies to those countries which co-operate and which have ratified the NPT. Carter has also, of course, been running down domestic development of reprocessing plants and plutonium breeders. Some nations well along the nuclear road have felt this legislation to be discriminatory and unreasonable, even to the extent of wondering whether the United States, whose breeder development was in any case lagging, was not adopting a pious stance based simply on commercial opportunism. In truth Carter's proliferation concern runs much deeper than that, but the last year has shown how unfortunate it is that commercial and national interests are so deeply woven into a fabric already criss-crossed with technological and international problems.

An idea put forward at the SIPRI meeting by A. R. W. Wilson, of Australia, could provide a way out of this bind. He proposes a nuclear fuel supply co-operative of states dedicated to meeting each others' fuel and service requirements whilst minimising the risk of proliferation. Each member would undertake to agree safeguards with the International Atomic Energy Agency (IAEA) and to restrict its access to enrichment and reprocessing facilities, whether on its own or another country's territory, simply to that amount of fuel deemed necessary by the IAEA for the country's power programme. Internationally controlled facilities would not be excluded from the co-operative's activities. The emphasis in the co-operative would be on mutual benefit rather than on surrender of national sovereignty.

This deserves extensive discussion. Much of the criticism of current non-proliferation measures is their sharply discriminatory character; Dr Wilson's proposal would represent a move towards benefit-sharing and should be widely attractive. □

The new exhibition schemes at the British Museum (Natural History) and the consequent changes in the more traditional exhibits have caused some consternation amongst interested scientists. At the recent symposium on Vertebrate Palaeontology and Comparative Anatomy held at Reading, Dr R. S. Miles, Head of the Public Services Department of the Museum explained the Museum's present policy towards its public exhibitions, and we present a summary of his talk below. Dr B. Halstead of the Department of Geology, University of Reading presents an opposing view (right).



A new exhibition on ecology (see above) opens this week . . .

The public's right to know

THE policy of the Public Services Department about what we do in the galleries is not the whim of any one person in the Natural History Museum. It originates with the 1753 Act of Parliament for the purchase of the Hans Sloane Collection. The British Museum Bill says: "The said Museum or Collection may be preserved and maintained, not only for the Inspection and Entertainment of the learned and the curious, but for the general Use and Benefit of the public." The Bill, in other words, distinguished between research and public functions for the museum.

What do we know about the visitors that come? The 'most common' age groups are children below 11 years of age; 26% are adults between 25 and 34 years of age. Of particular interest is the fact that children between 11 and 16 form only 3% of our visitors and young adults between 17 and 20 form only 11%. There is therefore some justification for regarding the Natural History Museum as a 'kids' museum, for children under 11. It appears that people come as young children, only to return when they are parents with their own children. So the vital years in education between 11 and 20 provide less than a quarter of our audience.

Approximately two-thirds of our visitors have completed their full-time education but eight out of ten have no qualifications in biology. Half of all visitors are visiting the museum for the first time and eight out of ten have not visited the museum on a previous occasion within the past year.

But in a summary of results in 1977 from our Annual Visitor Survey we found that many people set out to visit the museum with the expectation of learning something; when they left they had learnt practically nothing, or, more to the point, they gave no evidence of having learned anything, and not one that we interviewed had been stimulated to search out information for him or herself.

I think we are entitled to draw a few tentative conclusions from this. There is little evidence that we are fulfilling the Advisory Board for the Research Council's stated aim for the museum of attracting youngsters to science; and there is little sign of the university student who is supposed to make so much use of the public galleries in the museum. There would seem to be a distinct need for a new approach if we are going to fulfil the terms of the 1753 Bill.

A paper submitted to Trustees for their consideration in February 1972 initiated the new exhibition scheme. Entitled 'A Proposal for a New Approach to the Visiting Public', it starts by discussing the existing exhibition: "it is piecemeal and conceptually static, neglectful of natural processes and reflects to its loss the division of the museum into five separate departments." The 1972 paper says that the new

exhibition should deal with all forms of life, with the origin of the Earth and its life, and with the major geological processes which have affected life. It should point out areas of doubt and speculation and should complement the other national museums in London. It would have a uniquely comprehensive opportunity to display the full range of natural history. The paper suggests that the content of the new exhibition should be grouped under four headings: man, ecology, life processes and behaviour, and evolution and diversity.

There is no intention of reducing the number of organisms on display. On the contrary, the policy is to expand the exhibits illustrating diversity especially in invertebrates and plants which will be dealt with properly for the first time in the history of the museum.

My department of public services was created in January 1975 specifically to implement this new exhibition scheme. We want to make the museum an exciting place where the layman can enjoy exploring and discovering natural history. We need to attract an audience that is representative of the general public. We must recognise the need to motivate visitors.

Departmental policy gives rise to a long-term plan, which was represented by four documents written by museum scientists, members of all the departments. About 40 were involved in all and the four documents cover the four themes. The first three documents have been in the library of the museum since November 1972, the fourth, on evolution and diversity, since October 1973, and open for people to read and send in their comments. The evolution and diversity document, for example, says: "... the aim of the new exhibits on these topics should demonstrate the varied form and structure of species now existing; the historical relationships between them, and their status in the latest stage of continuing process of change on the surface of the Earth." These exhibits will contain the vast majority of the material now on show in the museum. The public will expect us to continue to provide what they have always found to be the most entertaining aspect of the museum—halls of monsters. On a more profound level, we hope the visitor will be able to derive an intellectually satisfying explanation of his origins, his unity with terrestrial life, and his uniqueness and his role. The exhibits will include Recent and fossil species and will be ordered systematically by relationships.

There is a problem to be solved: the diffusion of knowledge to the general public. It is not easily solved, but by a process of trial and error it is possible to develop better solutions. Our whole approach to the operation of mounting the exhibits is based on this assumption. □



... but should the museum stick to scholarly displays?

Whither the Natural History Museum?

IN his account of the thinking behind the new exhibition schemes, Dr Miles has posed clearly for the first time a fundamental dichotomy that exists in the concept of the role of the museum. The Department of Public Services has taken an unequivocal stand that its duty is primarily to serve the general public, and more than that, not to provide mere displays of the materials housed, but to communicate ideas and concepts.

The fundamental issue was summarised succinctly at the meeting by Professor T. S. Westoll. If one sees the role of the museum as one of social engineering, then one follows one route, by implication that of Miles' current policies. But if one sees it as a unique place in which to collect together in a systematic way what is contained in the natural world then clearly the national collection as a repository, with public galleries exhibiting those materials, will develop in a very different manner. It was evident that Miles' conception took no account of the needs of students but was aimed primarily at young people with no knowledge of biology and he saw his main task as one in which he would motivate them to learn. As a body, an audience of both professional and amateur palaeontologists, zoologists and geologists was in the main unsympathetic to Miles' thesis.

By far the most disturbing aspect of the discussion which followed Miles's talk was the strong impression of a serious breakdown of communication between the Museum's Department of Public Services and the scientific staff of the Natural History Museum, such that one of the world's leading ichthyologists on the museum staff was provoked to protest publicly at the recent removal of the fish exhibits to make way for the Human Biology Exhibition. It is known that the Museum's dinosaur expert would much have preferred a completely modernised Dinosaur Gallery, emphasising the natural history of these extraordinary creatures, rather than the entirely new exhibit, largely concerned with reptile relationships, which is now being constructed in the Central Hall. The museum has announced its forthcoming publication on the new dinosaur exhibit and yet its own expert is not involved in its production. This in spite of the fact that as well as being acknowledged as a world expert, he is one of the most experienced people around at communicating his subject to young people and the general public.

Dr H. W. Ball, the Museum's Keeper of Palaeontology, has publicly stressed on numerous occasions that the museum has the responsibility to curate, conserve and display the material objects in its charge.

It has always been assumed by people outside the museum, it now transpires mistakenly, that the public galleries reflected the responsibilities of the museum's

scientific staff working in conjunction with the exhibition staff. The fact that the new exhibition scheme seems to be going ahead in spite of serious opposition from the scientific side of the museum is surely a matter of deep concern.

The rigid dichotomy posed by Miles between the needs of the general public on the one hand and university students and teachers on the other is assuredly false. The late lamented dinosaur gallery was a case in point. It served the needs of the general public but also those of students. A still extant example of the same thing is the fossil mammal gallery. This scholarly exhibition is filled with genuine specimens with explanatory diagrams and models accompanying them. The essence of visiting this gallery is that one can see for oneself the actual evidence on which models and restorations have been based. Visitors have the possibility of deriving their own concepts, drawing their own conclusions. This exhibit can be visited at different stages in life, from a small child, to the general adult public and to advanced students. At each level something of real value can be gained.

Once it is conceded that it is an appropriate role for a national museum to be concerned with aspects of social engineering by promoting concepts that happen to be current in the present climate of opinion, then a most dangerous precedent is set, which has sinister implications. Suddenly it becomes possible to visualise museums contributing to the indoctrination of the more inarticulate sections of the community. Just so long as natural history museums are primarily concerned with displaying the materials in their charge, there is always the possibility that the facts will shine through the prevailing dogmas.

The new scheme as epitomised in the Hall of Human Biology leads to the grave suspicion that henceforth natural history specimens will become simply three dimensional illustrations of a given narrative aimed solely at "an audience that is representative of the general public". In my opinion the museum should be nearer to scientific literature than to the media.

The Natural History Museum's new exhibition scheme raises such fundamental issues that it deserves to have the fullest possible public discussion and for this to be effective it is essential that the views of the museum's scientific staff should be fully heard, together with those of teachers and students in schools and universities both here and abroad, who are intimately concerned with the signal service that the museum has provided in the past.

We can only hope that sufficient pressure can be brought to bear to curb the activities of the Public Services Department and to ensure the survival of the museum's reputation for scholarship in its public galleries. □



How problems in developing the Amazon region have boosted the status of environmental scientists



Brazil learns its ecological lessons—the hard way

The results of opening up the Amazon have been far from those intended: vast tracts of sterile land

SEVEN years ago Brazil's military rulers became the bogeymen of Western environmentalists when they announced that concern for the environment was a luxury of the industrialised countries which developing nations could not afford.

Today the military remains in power; but the message is very different. When President-elect Joao Baptista Figueiredo spoke recently of future development policies for the Amazon region, his references to the need for the "preservation of ecological equilibrium" to which permanent forest areas were "indispensable" could have been lifted from any environmental handbook.

The lesson has been learnt the hard way. Not primarily by listening to scientists and ecologists who, for the past 15 years, have been warning of the destructive effects of uncontrolled development on one of the world's major natural resources; but from experiencing the failure of ambitious plans to "recolonise" the region using techniques developed elsewhere and ill-suited to local conditions.

The result has been a growing awareness in official circles that the environmental impact of development programmes cannot be ignored. "In the past few years all sides have begun to see that poor environmental control can create bottlenecks in economic development," says Dr Paulo Nogueira Neto, a former biology professor who is now head of the environmental protection division of the Ministry of the Interior.

A by-product has been an increased willingness to involve environmental scientists, to whom government planners are now turning as they realise that successful development requires,

inter alia, a firm grounding in detailed knowledge of the behaviour of biological systems and their component parts.

The price of failing to respect such systems has been dramatically illustrated by the effects of the 14,000 miles of highway that have been driven through the Amazon forests like a military exercise over the past decade. The purpose of the roads has been both to open up trade with the continent's west coast, and to encourage the "recolonisation" of the region by settlers who would, it was hoped, convert the biological richness of the forest lands into valuable primary products.

Many of the results have been far from those intended. The roads themselves, disrupting streams and rivers and destroying delicate ecological balances, have produced vast tracts of sterile land. And the settlers, after a year or two of intense cultivation on land from which the trees had been removed, found agricultural yields drop sharply as nutrients were leached from the soil, and soon left to return to the urbanised south.

Thus has developed a practical awareness that, despite all its apparent robustness, the tropical forest is a fragile system in a state of precarious equilibrium; "a desert covered by trees" as it has been called by ecologist Robert Goodland. Less than a fifth of the Amazon region contains productive soil, and agricultural techniques developed in temperate zones have shown themselves frequently inappropriate to tropical conditions.

"The road was a school for us. You have to pay for everything that you do, and Brazil has paid quite a lot for its mistakes," says Dr Antonio Machado, programme director of the National Research Council's humid tropics pro-

gramme, a 3-year \$15 million project on the use of forest resources partly financed by the International Development Bank.

"But although we learnt by the wrong way, we now know much more about the soil, the forests, the water and the weather, and the effects on these of human settlements. And with this information we now have enough to start trying to exploit the forest in a rational way".

One research centre which has been brought into prominence by the new-felt need for scientific knowledge is the National Institute for Amazonian Research (INPA). Founded 20 years ago and financed by the National Research Council, the institute is set in 45 acres of deliberately-untouched secondary forest on the outskirts of Manaus, the main trading town on the Amazon river 1,600 kilometres from its mouth.

Much of INPA's work, like that of the older Goeldi Museum in Belém which is now part of the institute, continues a tradition of study of the unique biological systems of the Amazon region that stretches back through Charles Darwin to Alexander von Humboldt. And previous government antagonism to the opinions of scientists stemmed from the fact that many of the latter tended to see a direct conflict between their interests in "undisturbed" natural systems and the goals of development programmes.

Under its present director Dr Warwick Kerr, who was appointed three years ago on secondment from the University of São Paulo, attitudes have changed. Dr Kerr's outspoken views on social issues have frequently ruffled feathers in the military hierarchy, but he is committed to the belief that INPA's activities must be directed towards the well-being of the people of the Amazon region—part of a com-

promiso which he claims all research institutions have with the society that supports them.

Dr Kerr warns that at current rates of exploitation, the Amazon region's 2.7 million square kilometres of forest would disappear in less than 30 years, and that the urgent need, to which scientists must contribute, is for a pattern of controlled development that takes into account both the potential and the vulnerability of the region's resources.

Referring to the fertile *várzea* regions which receive their nutrients from annual flooding, for example, Dr Kerr says: "the floods, instead of being sources of discomfort, should be looked upon as a blessing and a source of wealth. We should learn how to cope with them, developing systems of housing, of experimentation with livestock, of conserving the forests essential to both fish and to man, and better systems of river transport".

INPA's activities reflect this commitment. There are now over 30 research scientists working at the institute—when Dr Kerr was appointed his arrival is said to have doubled the number of PhDs from one to two—and the work is divided into six divisions: biology, medical sciences, parasitology, agronomy, fish and fishing, and technology.

Each division combines both basic and applied research. In the fish section, for example, research topics range from fish biology to the life-cycle of the manatee, once a plentiful source of meat but now almost extinct through over-hunting; and the agronomy section includes both Dr Kerr's work on the genetics of bees and methods of soil management.

INPA also runs an annual programme of "intensive training" for work in the Amazon. The course, which lasts six months and currently has about 40 students, combines a study of Amazonian ecology and tropical hygiene with groundwork in research methodology (including statistics) and general laboratory techniques.

The growing concern of both local and federal development agencies for the environmental aspects of their programmes has led many to consult INPA for advice. And such interaction has helped INPA to develop a working relationship with government agencies which had initially been suspicious of its attempts to broaden the scope of its activities.

Thus for some time there has been tension between INPA and the Brazilian Agricultural and Ranching Research Bureau (EMBRAPA) over a mutual interest in agricultural research for the Amazon region. Much of this tension was removed, however, by a meeting of the staffs of the two in-

stitutions at the beginning of August, where hostility was transformed into an enthusiasm for cooperation, although with general agreement that INPA should remain responsible for the more basic aspects of the research.

A commitment to "useful" research has not been allowed to swamp more traditional interests of long-term value in the fauna and flora of the Amazon region. A major programme of theoretical ecological studies is being carried out under the direction of Dr Herbert Schubart. And a new venture, which is being organised jointly with the US-based World Wildlife Fund, involves investigating the minimum area that can be isolated as a self-contained ecosystem retaining a full range of biological and genetic diversity.

A local agency responsible for developing methods to exploit the forests resources has agreed to set aside 62 plots of land, ranging in size from one-tenth to 10,000 hectares, for the study. Before the plots are isolated and regularly thereafter for a number of years inventories of the species found on each plot will be compiled by research workers from INPA. "The preservation of the tropical rain forests and the life-forms it contains is a number one priority in our attempts to protect the biological diversity of the planet," explains Dr Thomas Lovejoy, programme scientist of the WWF, a frequent visitor to INPA, and principal investigator of the project.

Both the WWF and INPA have also been contributing to plans drawn up by the Brazilian Institute for Forestry Development (IBDF) to create a series of nature reserves — "conservation units"—in the Amazon region, which at present contains only one of Brazil's eight national parks. The areas recommended as reserves have been carefully chosen by drawing on the scientific records of the critical habitats of different species, and identifying the areas of the Amazon

where these overlap.

The government, partly under pressure from international bodies such as the United Nations Education and Cultural Organisation (Unesco), has already shown itself more willing than in the past to incorporate such proposals in its development plans. A major problem, however, is that much of the land in Brazil is privately owned, and thus outside direct government control.

"The government attitude has certainly been much better than previously. But our main problem is that although we already have eight national parks throughout Brazil covering almost two and a half million acres, less than half a million acres belongs to the government," says Dr Maria Padua, head of the IBDF team that drew up the "conservation unit" proposals.

In the Amazon region, despite attempts to stimulate the development of small-scale enterprises, most of the running has so far been made by large private enterprises. Volkswagen de Brazil, for example, has bought 300,000 acres of forest which it has burnt down for conversion into a giant cattle ranch; and near the mouth of the Amazon an elusive US billionaire, Daniel K. Ludwig, has bought a million acre estate, cut down much of the forest to grow rice and installed a \$300 million Japanese pulp mill.

Dr Kerr's wish for a controlled development that meets the needs of the Amazon people therefore takes on a special meaning. Brazil's growing ecology movement, drawing on biological and environmental arguments, favours a development strategy based on small, relatively self-sufficient communities, allowing the forest to produce a sustained yield of natural products; economic pressures, however, remain weighted towards large-scale enterprises primarily seeking high-level, often short-term, return on investment.

Infra-red telescope will not be ready for Jupiter mission

ONE of the things in life that even the National Aeronautics and Space Administration has been unable to speed up is the hand-finishing of large telescope mirrors. Consequently a current source of embarrassment to the agency is a last-minute delay in completing work on a 120-inch mirror to be installed in its infra-red telescope at Mauna Kea in Hawaii.

The telescope has been commissioned by NASA at a cost of \$6m, and will be used for ground-based observations of planets and other bodies in the solar system to complement data gathered

by spacecraft.

Its first task was to have been to pin-point the position and movement of "hot-spots"—thought to be gaps in the cloud which reveal the higher temperatures, in the atmosphere beneath—on the surface of Jupiter. This information would have been used by the two Voyager spacecraft to direct infra-red sensing equipment and plan the sequencing of operations to gather scientific data.

The two spacecraft were launched last year on courses that take both past Jupiter, and Saturn, and Voyager

It is due to reach Jupiter early next March. However, delays in finishing the mirror, which is being prepared in the workshops of the Kitt Peak Observatory in Arizona, mean that the telescope is not likely to be ready until the end of that month, and completion has been officially scheduled for the beginning of April.

NASA is now planning to rely on the 200-inch Mount Palomar telescope as well as an 88-inch infra-red telescope in Hawaii, to make the necessary observations both prior to and during Voyager 1's fly-past. But officials admit that the quality of the data will be inferior to that eventually expected from the 120-inch telescope.

The delays in the new mirror came first in developing new computer-based procedures for testing the quality of the mirror; and when these procedures were recently completed and the tests applied, it was found that an additional three months' work was still required.

"We are doing all we can to speed things up. But it is the kind of delicate work that does not lend itself to working in shifts; you need a complete crew that is in tune with the project and with each other", Mr Norman Cole, master optician at Kitt Peak, said last week. Mr Cole has also been responsible for the mirrors not only of the 4-metre telescopes at Kitt Peak itself and at Cerro Tololo in Chile, but also of an 84-inch infra-red telescope built by the University of Wyoming with funds from the National Science Foundation, which came into operation last year.

The team preparing the mirror has eight members, and polishers work in the final stages by removing only a few millionths of an inch at a time from selected spots. Mistakes are difficult and time-consuming to correct: "If you go overboard in one touch, then it takes time to blend it back into the surface", explains Mr Cole.

Scientists at NASA's Jet Propulsion Laboratory in California are disappointed that the Mauna Kea telescope will not be ready for the experiments that they plan to carry out when Voyager 1 passes Jupiter, but are confident that the other two telescopes will produce adequate data, and that Mauna Kea will be ready for Voyager 2's fly-past in July.

However the delay is likely to lead to questions in Congress, which was told in 1974, when considering an initial funding request for the telescope, that "no existing telescope" was able to provide the data necessary for planning the Voyager missions, and was assured earlier this year that construction was on schedule.

Dr Bill Brunk, NASA's head of planetary astronomy, admitted last week that the situation was an "embarrassing" one for the agency; "I can only answer that we tried our best to finish it on time". **David Dickson**

Saudi Arabia invests in fishing

THE Red Sea is one of the poorest seas for fishing in the world. It is formed by a deep narrow trench making it very poor in nutrients. In the few areas where there are fish a specialised fishing industry has never been developed because of the many varieties of fish. The Saudi government, however, is trying to modernise the local fishing industry and introduce fish farming along the Red Sea coast. The scheme is being conducted by the Ministry of Agriculture and Water Resources.

In 1976, the ministry signed an agreement with the British White Fish Authority for a four year cooperation programme. So far the programme has assessed the fish resources of the Red Sea and the Persian Gulf and has studied catching methods and ways of increasing fish landings. An aerial survey has shown that there are 72 fishing communities on the Red Sea coast with a fleet of 1,225 boats. Most of these boats are traditional "dhows" which are rather inefficient. The estimated annual landing is 10,000 tons.

Any new additions to the fishing fleet must be easy for local fishermen to use and for local shipyards to maintain. The design criteria have been met by the Cornish boatbuilders Cygnus Marine who have already produced two vessels on an experimental basis. Initial indications are that these small, 10m fishing vessels will play a major role in the fishing industry of Saudi Arabia. A fleet of 25 such boats could increase the catch in the Gulf by 25%.

The Ministry of Agriculture and Water Resources also operates a research vessel, *Ibn Majid*, which has facilities for all forms of fishing. It has

discovered new fishing grounds in the south where there are more nutrients.

The modernisation of the Saudi fishing fleet, however, does not solve the Saudi fishing industry's basic problem—the lack of fish in the Red Sea and the Gulf. Consequently, the idea of fish farming has gained considerable ground. Normally, fish farming is expensive; in Saudi Arabia, however, four factors work in its favour:

- the large market for fish.
- the abundance of unpolluted sea water. Despite an increase in pollution after the re-opening of the Suez canal, the Red Sea remains one of the cleanest seas in the world.
- the abundance of cheap energy for pumping water through fish ponds.
- fish farming would transform large arid coastal plains with no other agricultural potential into food producing areas.

areas

The Fishery Development Laboratory in Jeddah has studied soil samples from probable locations for fish farms. A large area of the Saudi coastline is suitable for the pond/tank method of fish farming. Fish are reared and fed in fenced-off sea pens.

The farms would consist of 25m long ponds perpendicular to the line of the coast. Clean water will be pumped from beyond the reef edge to a header channel from which it will flow by gravity through the ponds before discharging into a channel which runs back to the sea. The ponds will be designed to farm three species: mullet, rabbit fish and a local favourite known as "Bouliti". Fish farming is expected to produce 829 tons of whole fish a year.

Ziauddin Sardar

Stevenson to introduce new DNA legislation in US

SENATOR Adlai Stevenson, chairman of the Senate Subcommittee on Science, Technology and Space, has announced that he intends to introduce new legislation covering research involving recombinant DNA techniques soon after Congress reassembles next January.

According to Stevenson, even though "public opposition to the research has virtually disappeared" as a result of federal actions taken so far, a number of reasons remain to make legislation advisable. These include the fact that federal support of research on

the risks of recombinant DNA techniques will be necessary to keep pace with the development of the techniques themselves; that it remains "anomalous" to restrict one class of researchers but not another, merely on the basis of their location and source of funds (current guidelines now under final revision by the National Institutes of Health would apply only to NIH-funded research); and that "a programme of voluntary compliance by and monitoring of industrial research activities (as proposed in the revised guidelines) is insufficient". □

The Nobel Prizewinners 1978: Physics

A sensitive ear for the Big Bang

Michael Rowan-Robinson writes:

A MODEST and laconic letter entitled "A measurement of excess antenna temperature at 4080 Mc/s" reached the *Astrophysical Journal* on 13 May, 1965. The paper, only 600 words long, describes in a brief, almost brusque, style the first project undertaken by two young radioastronomers since their move to Bell Telephone Laboratories. Using a 20 ft horn reflector built by Bell Labs engineers for the "Echo" satellite project, they had measured the temperature of the antenna when pointing in the zenith (vertical) direction. They had found a temperature of 6.7 K (degrees Kelvin, or absolute), of which 2.3 K was due to atmospheric absorption and 0.9 K was due to Ohmic losses in the antenna. This left 3.5 ± 1.0 K unaccounted for. The two astronomers had worked very hard to eliminate noise sources in their antenna, but could not explain the excess.

Had this paper appeared in the *Bell System Technical Journal* like the other work with the horn antenna, astronomers might hardly have noticed it, as they hardly noticed Jansky's earlier discovery at Bell Labs of radio emission from the Milky Way. Penzias and Wilson did not themselves immediately recognise the significance of their discovery. However they had the good fortune to be near Princeton, where the group led by Dicke and Peebles were only too aware of the significance of a 3 K background. In early measurements with the radiometer which he invented, Dicke had noted in 1946 that the temperature of any cosmic background radiation was certainly less than 20 K. He had then turned to other experiments and to the development of the Brans-Dicke cosmology. By 1965 Dicke, in collaboration with Peebles, had independently reinvented the hot Big Bang model of Gamow, was predicting the existence of cosmic microwave background radiation with a blackbody spectrum (in seminars if not in print) and had two students, Roll and Wilkinson, building a system to measure the temperature of this background.

The plans and dreams of the Princeton group must have been rudely shattered by the news of Penzias and Wilson's discovery. The four of them wrote a beautiful paper explaining the significance of the radiation as the relic of the "primeval fireball" phase of the hot Big Bang, and outlined the physics of this radiation-dominated, opaque



Arno A. Penzias

"To Dr Arno A. Penzias and Dr Robert W. Wilson, Bell Telephone Laboratories, Holmdel, New Jersey, USA for their discovery of the cosmic microwave background radiation."



Robert W. Wilson

phase of the universe's history. This paper appears immediately preceding the one by Penzias and Wilson, with the far more dramatic title "Cosmic blackbody radiation".

Penzias and Wilson included only one very cautious remark on the significance of their 3 K background: "A possible explanation for the observe excess noise temperature is the one given by Dicke, Peebles, Roll and Wilkinson in a companion letter in this issue". Penzias had heard about the Princeton group's ideas and invited them over to Holmdel. Without that visit the only reference to the excess noise might have been the one that

appears in a paper by Penzias and Wilson on the flux from the radio source Cas A, received in its original form by *Astrophysical Journal* on 1 April, 1965: "The equivalent temperature of the antenna when pointed at the zenith is about 7 K, 2.3 K of which is due to absorption by oxygen in the atmosphere".

While it is probably inevitable that Dicke and his group would have discovered the cosmic blackbody radiation within a year or so, it was also inevitable that Bell Labs would sooner or later identify any source of background noise in the microwave region. Earlier they had, through Jansky, identified the effect of the Milky Way in the radio region. It is taking nothing away from Penzias and Wilson to say that part of the credit for their discovery must go to the thorough, professional and single-minded work of the Bell Lab scientists and engineers over the years in developing sensitive receivers and reliable antennae. As Penzias and Wilson note in their historic paper, the 3 K excess is implicit in earlier work by Bell Labs scientists with the same antenna (Degraesse, Hogg, Ohm & Scovil).

The discovery of the microwave background radiation came early in the careers of Penzias and Wilson. It was Penzias's third paper and Wilson's fourth. Subsequently they measured the temperature of the background at 21 cm and 2.6 mm and set limits on its large- and small-scale anisotropy, but the bulk of their later work has been in microwave molecular line astronomy. Penzias has exerted a very considerable influence on the development of this field, always with Wilson's brilliance with instrumentation at his right hand. In collaboration with Jefferts, also of Bell Labs, they discovered the most important interstellar molecule apart from hydrogen—carbon monoxide—and with him and other collaborators they were responsible for the discovery of microwave line emission from 6 other interstellar molecules: CN, SiO, CS, CH₃CN, OCS, and H₂S in the early 1970s. In the past 5 years their main interest has been in the relative abundances of interstellar isotopes and whether these vary from place to place in the Galaxy. They have recently designed and built a novel millimetre-wave telescope. Typically, Bell Labs insisted that the dish be built in New Jersey, rather than at some astronomically more favourable, high-altitude site, Bell Lab's interest in

the telescope is for communication with other human beings rather than with the cosmos.

One curious feature of the story of the discovery of the cosmic blackbody radiation is the ignorance of the scientific literature of all concerned. Neither the Bell Labs nor Princeton workers seem to have been aware of Gamow's idea of a hot Big Bang (*Nature* **162**, 680; 1948) or the prediction of Alpher & Herman in 1948 of a background temperature of 5 K (*Nature* **162**, 774; 1948). More forgivably they had not noticed McKellar's 1941 calculation that interstellar CN absorption line ratios implied a CN

excitation temperature of about 2.3 K (Publications of the Dominion Observatory **7**, 251).

When the Nobel committee opened the doors to astronomy with the 1974 award to Ryle and Hewish, many of us felt that it must be only a matter of time before the discovery of the cosmic blackbody radiation was similarly rewarded. It was one of the astronomical discoveries of the century, transforming cosmology into an exact science and demolishing the already shaky Steady State Theory. The blackbody nature of the spectrum is qualitatively confirmed by the most recent balloon-borne experiment (Woody & Richards,

Phys. Rev. Letters, to be published) and now interest focuses on possible distortions of the spectrum. The isotropy of the radiation both on the large and small scale is well-established apart from the small effect of the Earth's motion in space (see *Nature* **270**, 9; 1977). While we must spare a thought for Dicke and Peebles, who came so close to making this discovery, and who contributed so substantially to its interpretation, and for Jansky, who must surely have shared one of these astronomy prizes had he lived, there is no doubt that the Nobel committee have chosen deserving prize-winners. □

Fifty years of scientific achievement

David Shoenberg writes:

THE "basic inventions and discoveries in the area of low temperature physics" for which Peter Kapitza has been awarded a Nobel Prize in physics were essentially completed over 30 years ago. His many friends and admirers will, however, regard the award as a well merited and, indeed, long overdue recognition of over 50 years of scientific achievement in a much wider field and of the inspiration and guidance he has provided for many generations of physicists in England and the Soviet Union.

As a young man in his mid-twenties he was one of the enthusiastic group led by A. F. Ioffe in Leningrad who were building a school of Soviet physics in the difficult days following the Revolution and its aftermath of civil war and famine. He was already known for his originality and ingenuity in solving practical problems and he was chosen as one of a delegation sent in 1921 to establish scientific contacts with the West.

Kapitza came to Cambridge and was very keen to work under Rutherford in the Cavendish Laboratory. Legend has it that Rutherford was rather cool at first and said the Cavendish was full, to which Kapitza replied by asking what Rutherford regarded as a reasonable experimental error in his work. When told that it was usually 2 or 3%, Kapitza triumphantly pointed out that since there were 30 or so researchers, one more would be just about within the margin of error and this ingenious argument persuaded Rutherford to admit him.

During the 13 years he stayed in Cambridge he became somewhat of a legend not only for his scientific achievements but for the broad sweep of his ideas, his love of argument, his

'To Professor Piotr Leontevitch Kapitza, Institute of Physical Problems, USSR Academy of Sciences, Moscow, for his basic inventions and discoveries in the area of low temperature physics.'



Piotr Leontevitch Kapitza

enormous repertoire of anecdotes (recounted with great verve in a species of broken English which was entirely his own), his absentmindedness and above all his personal charm. At his informal weekly seminar, the Kapitza Club, as it was called, he loved to stir up discussion by asking provocative questions and often new ideas would be thrown up which later proved important.

After some early work on nuclear

physics, Kapitza took up the study of physical properties in very high magnetic fields and at very low temperatures. He developed a novel technique for obtaining fields of over 30 T by discharging a considerable energy into a coil during only 1/100 s, a time short enough to avoid much Joule heating. He liked to boast that he was probably the highest paid physicist in the world since his total work in a year lasted only a few seconds. To attain the low temperatures, he invented a new kind of helium liquefier in which the bulk of the cooling was provided by adiabatic expansion. The difficulty of lubricating the expansion engine at low temperatures was avoided by using a rapid stroke and effectively using the gas as a lubricant. Kapitza's machine was in fact the forerunner of a liquefier developed on similar principles, but for factory production, by Sam Collins at MIT. The Collins liquefier opened up the field of low temperature physics to anyone with money enough to buy a machine and so Kapitza's invention was in a sense responsible for the explosive growth after the war of low temperature physics all over the world but particularly in the United States.

In 1934, soon after the opening of the Royal Society Mond Laboratory, which had been built to house Kapitza's equipment and just as he was preparing to exploit his techniques systematically his work in Cambridge was brought to an abrupt end. During a routine visit to Russia he was unexpectedly refused permission to return to Cambridge and told that he must work in Russia instead. In spite of urgent pleas by Rutherford and diplomatic interventions at the highest level, the authorities were adamant and eventually Kapitza had to submit and

go through the wearisome process of designing and equipping a laboratory all over again. In this he was greatly helped by Rutherford, who persuaded Cambridge University to sell much of the specialised equipment (or duplicates of it) to the new Institute for Physical Problems, as it was called, and Kapitza was able to start work again early in 1937.

Soon after the Institute was operational Kapitza took up the study of the remarkable properties of helium II, the phase of liquid helium below the λ -point (2.18 K). Keesom's discovery in Leiden that it appeared to be a "super-heat-conductor" had caused great interest and in 1938 Kapitza in Moscow and Jack Allen in the Mond Laboratory in Cambridge simultaneously discovered that helium II was also a "superfluid" which could flow through narrow apertures without any driving pressure head. Eventually further experiments, particularly on the thermal effects associated with flow, led to the idea that helium II was a kind of intimate mixture of a "superfluid" which moved completely freely but carried no entropy and a "normal fluid" which did carry entropy and suffered viscous resistance to flow.

This "two fluid model", originally proposed on a semi-empirical basis by Tisza, was a little later more fundamentally established by Landau quite independently, as part of the basic quantum theory of superfluid helium he developed. [Landau was the head of the theoretical group in Kapitza's Institute and his theory proved to be seminal since it not only explained the existing experiments, but predicted several exciting new effects, which were looked for and found under Kapitza's leadership.] It was mainly for this theory that Landau was awarded a Nobel Prize in 1962.

For over 40 years the Institute for Physical Problems has under Kapitza's lively guidance continued to be in the forefront of low temperature research, particularly on superconductivity and the dynamics of electrons in metals as well as on the behaviour of liquid helium.

Kapitza himself, however, has turned to other fields. One technique to which he has made major contributions is the use of expansion turbines to liquefy air on an industrial scale in order to separate oxygen by fractional distillation. But his major activity during the last 25 years and still continuing to-day has been the use of high power microwaves to heat plasma with a view to providing the right conditions for nuclear fusion.

It is remarkable that at 84 he is still as vigorous and enthusiastic as ever. Perhaps another Nobel Prize is yet to come. $\square$

The Nobel Prizewinners 1978: Medicine

From modest beginnings . . .



Hamilton O. Smith, Daniel Nathans and Werner Arber

'To Dr Werner Arber of the University of Basel, Switzerland and Dr Daniel Nathans and Dr Hamilton O. Smith of Johns Hopkins University, Baltimore, Maryland, USA for the discovery of restriction enzymes and their application to the problems of molecular genetics.'

Richard Roberts writes:

THE restriction endonucleases, which have become so familiar to the molecular biologist, have finally come of age with the award of this year's Nobel Prize in Physiology and Medicine to Dr Werner Arber of the University of Basel and to Drs Daniel Nathans and Hamilton O. Smith of Johns Hopkins University. They each played a critical but separate role in drawing attention to these bacterial enzymes which dominate so much present research. Amid the proliferation of sophisticated techniques which characterise modern molecular biology it is the restriction endonucleases more than anything else which have made possible the dissection and analysis of the very large eukaryotic genomes, have allowed spectacular advances in DNA sequence analysis and have triggered the important field of recombinant DNA research. The recent major discoveries of overlapping genes and RNA-splicing were made with the assistance of restriction enzymes and it is becoming increasingly difficult to imagine how it was possible to do without them.

The first hints that restriction enzymes existed came in the early 1950s when Dr S. Luria and his colleagues reported a phenomenon they termed host-induced variation and which is now known as host controlled restriction-modification. They observed that bacteriophages grown in one strain of bacteria were often able to grow only poorly in a second strain. The few progeny emerging from the second strain, however, were able to grow

perfectly normally upon reinfection of the second strain, but now grew poorly upon the first strain. This phenomenon attracted the attention of Dr Werner Arber and by 1962, he and a colleague D. Dussoix, had conducted a series of elegant genetic experiments which provided impressive evidence concerning the nature of this "variation". Together they proposed a molecular mechanism to explain host controlled restriction-modification.

They postulated that certain bacterial strains contained an endonuclease (restriction enzyme) which was able to cleave unprotected DNA, and furthermore that those same strains must contain a strain-specific modification enzyme responsible for the protection of the cell's own DNA from the action of the restriction enzyme. Upon infection by an unprotected bacteriophage the phage DNA was destroyed by the restriction enzyme, except for some small fraction which was modified before encountering the restriction enzyme. Such modified phage were then able to grow perfectly normally upon reinfection of this same strain.

Following the detailed characterisation of the biological phenomena, both in Arber's laboratory and others, this system was analysed *in vitro*. In 1968 Arber and his colleague S. Linn, demonstrated the activity of the *Eco* B restriction enzyme and M. Meselson and R. Yuan purified a similar enzyme from *E. coli* K. It is now known that these Type I restriction enzymes recognise a specific sequence of nucleotides within a DNA molecule, but

cleave the DNA at random positions often far removed from the recognition site. Because of the random cleavage these Type I enzymes have not proved valuable as tools of the molecular biologist. Arber's studies, however, set the stage for the discovery of a second type of restriction endonuclease.

In 1970, Dr Hamilton O. Smith and his colleague K. W. Wilcox, described the outcome of experiments initially aimed at studying recombination in *Haemophilus influenzae*. The introduction to their paper states, "We made the chance discovery of what appears to be a similar enzyme (to the Eco B and K enzymes) in *Haemophilus influenzae*, strain Rd". Thus was described the isolation of the very first Type II restriction enzyme in a discovery destined to revolutionise the practice of molecular biology. In an accompanying paper, written with a colleague T. R. Kelly, it was shown that this enzyme called *Hind* II both recognised and cleaved a specific sequence of nucleotides within a DNA molecule. It thus resembled the Type I enzymes in its recognition properties, but differed significantly in its cleavage properties. It is this difference (the specific cleavage induced by the Type II restriction enzymes) which is responsible for their value to the molecular biologist. The methods used by Smith and his associates to purify *Hind* II and to characterise its recognition sequence have proved generally useful and the majority of the 150 or so restriction enzymes now known to exist have been purified and characterised in a similar way.

From our present perspective it would seem a natural and obvious experimental path to use a specific endonuclease such as *Hind* II to prepare discrete fragments of a DNA genome. However, in the late 1960s gel electrophoresis, a technique critical to current experiments did not enjoy its present popularity. Arber and Smith's experiments used sucrose density gradients or viscometric assays to characterise the products of restriction enzyme digestion, techniques which mask the exquisite specificity of the cleavage products. We must also remember that the major advances in molecular biology had been made in prokaryotic systems where a wealth of genetic back-up had been indispensable to progress. Such was not the case for the small tumor virus SV40 which had just begun to attract the attention of the molecular biologist. Already it was clear that the approaches used for the study of bacteriophages, were not so easily applied to the study of SV40.

Among those studying SV40 in these early days was Dr Daniel Nathans, who worked in the same department as

Smith. After learning of the interesting and unusual properties of *Hind* II and realising the significance of the specific cleavage he set about examining the effects of this enzyme and the enzymes from *E. coli* upon SV40 DNA. Among the techniques chosen to examine the cleavage products was polyacrylamide gel electrophoresis. The results of these studies, carried out with his colleague K. Danna, were published in 1971 and the first gel showing the *Hind* II fragments of SV40 DNA revealed the intrinsic power of the restriction enzymes to allow access to the secrets of the DNA genome. For Nathans had correctly realised that the restriction enzyme cleavage sites could serve as reference points for mapping and could thus replace the conventional genetic markers. In a continuing series of

elegant experiments Dr Nathans has taken advantage of the many facets of restriction enzymes to further his studies of SV40.

As so often happens in science a rather slow and modest beginning suddenly leads to an explosive burst of interest and a rush of new knowledge. Arber's painstaking studies leading to the formulation of the molecular mechanism for host controlled restriction-modification allowed Smith to appreciate the nature of the enzyme activity he discovered in *H. influenzae*. The potential value of this kind of enzyme as a tool for research was realised by Nathans. Today it is scarcely possible to pick up a biochemistry journal without being inundated by the restriction enzyme acronyms. □

Mary Lindley writes from Baltimore:

THE excitement that spread through Johns Hopkins University last week was mixed with a hint of relief that this august institution had its very own Nobel Prize at last. Although other Nobel laureates have been associated with the university, Daniel Nathans and Hamilton O. Smith are the first incumbent members of the faculty to receive the prize.

Unlike almost all other Nobel prize-winning scientists, neither Smith nor Nathans are members of the US National Academy of Sciences. Neither was aware that their work had been nominated for the Nobel Prize, and their first response to the news, received by telephone early in the morning of 11 October, was a mixture of shock and disbelief. After the initial surprise had subsided, however, they were quick to acknowledge the contributions of the colleagues who had helped and influenced them during crucial stages.

Smith recalled how he and a graduate student, Kent Wilcox, became interested in restriction enzymes in 1968, while working on bacterial recombination. In the course of that work they incubated the bacterium *Haemophilus influenzae* with DNA of the bacterial virus P22, and were puzzled by finding that the DNA was broken up. Smith then recalled the work of Werner Arber in Switzerland, who had laid the genetic foundations for an understanding of the process whereby bacteria can destroy foreign DNA (and who now shares the Nobel Prize). And he thought also of M. Meselson's report of a restriction enzyme produced by the bacterium *Escherichia coli* K that could attack and break down foreign DNA.

Smith and Wilcox then set to work to look for such an enzyme in *Haemophilus influenzae*. By the spring

of 1969 they had found and purified it. But then Wilcox was drafted into the army, leaving Smith to continue the work alone. He progressed with much help from Bernard Weiss, working in a neighbouring laboratory, and later with the vital collaboration of Thomas J. Kelly Jr, newly graduated from Johns Hopkins University Medical School. Smith and Kelly soon identified the specific site on the DNA where the enzyme made its attack.

Meanwhile Smith had written to his friend Nathans, then working in Israel, about the interesting enzyme he had found in *Haemophilus influenzae*. At that time Nathans was interested in the relatively unexplored molecular biology of the virus SV40. He recalled that he had been wondering how he could obtain pieces of its DNA. Smith's letter provided the answer. He realised that restriction enzymes that attacked specific sites could be used to manipulate DNA in the same way that the enzymes trypsin and chymotrypsin were used already to analyse proteins.

Back in his laboratory in Baltimore, early in 1970, Nathans confirmed that restriction enzymes could attack the DNA of SV40. Then, together with colleagues K. J. Danna and G. H. Sack Jr, he set about using them to investigate the structure and function of that viral DNA. The work made rapid progress, and eight years later restriction enzymes were among the hottest properties in molecular biology. And then came the early morning phone call.

Of the contributors to this section, Michael Rowan-Robinson is at Queen Mary's College, London, David Shoenberg is at the Mond Laboratory, Cambridge, Richard Roberts is at the Cold Spring Harbor Laboratory, New York and Mary Lindley is on sabbatical from Nature at Johns Hopkins. A report on this year's prize-winner in chemistry, Peter Mitchell, follows next week.

news and views

Are deserts ecosystems?

from Peter D. Moore

THE second International Congress of Ecology (INTECOL) was held recently in Jerusalem* and the choice of venue made it inevitable that some emphasis would be placed upon desert ecosystems. Or should one refer to the deserts as ecosystems at all? N. Meir (Hebrew University, Jerusalem) introduced a controversial, if semantic, note early in the proceedings by stating that since interaction between species is, of little importance in desert communities, the understanding of desert ecology involves simply the sum of autecological information about the component species. The ecosystem concept, he claimed, is of little value in such habitats. This led to the impromptu construction of a desert ecosystem model by H. T. Odum. Evidently, it is still respectable in some quarters to regard deserts in ecosystem terms.

Meir's point (originally made by Darwin) about the low level of interactions between species in deserts, however, cannot be disputed. Plant density is low, mortality is largely density independent, pollination mechanisms are largely unspecialised and mycorrhizal relationships are rare. Seed predation can be very heavy on the other hand; often 90% of a plant's seeds may be eaten by rodents. The animal/plant interaction may be delicately balanced and plant species which are particularly dependent on seed production (r-strategists) may have their populations controlled by seed predators. S. R. Morton (University of California, Irvine) pointed out that there is about three times the number of granivorous mammal species in the North American deserts than in the deserts of Australia. Seed predation in Australia is still very high, however, but birds and ants frequently assume this trophic role. He suggested that this could be due to the late (Oligocene) arrival of rodents in Australia which

would have permitted the diversification of birds and ants into the available niches.

The main constraints on the flora which prevent competitive interactions developing are evidently the high temperatures and lack of water in the deserts and some accounts were given of remarkable adaptations to these extreme habitats. For example, there are the endolithic microorganisms found within porous rocks, such as sandstones and limestones. E. I. Friedmann and R. Ocampo-Friedmann (Florida State University) described such organisms from both the cold deserts of the Antarctic and the hot deserts of Sinai. The outer layer of rock, about 1 mm in thickness, is usually devoid of living matter, but its pores are often blocked by debris. The next 1 mm layer is occupied by photosynthetic algae (often with fungal associates in a primitive lichen symbiosis) or by cyanobacteria or colourless bacteria. Nitrogen is generally not fixed by these organisms, but is available from the rock or from atmospheric fixation. The blockage of pores in the outer layer effectively seals the inner system, leading to water conservation. The advantage of such a closed hydrological system is evident when one considers the data of L. Kappen and O. L. Lange (University of Würzburg) who have shown that some fruticose desert lichens, such as *Ramalina massiformis* demand relative humidities of greater than 90% before there is a measurable net gain in CO_2 . This means that only in a brief period after dawn, following nightly dew absorption, is there a net photosynthetic gain. Such a species can only achieve an annual relative growth rate of $195 \text{ mg CO}_2 \text{ g}^{-1} \text{ dry weight}$. Hiding in the rocks has its advantages.

P. J. Weisser (University of Chile, Santiago) described a rather similar site adaptation in a species of the cactus genus *Thelocophala* from the Atacama desert of Chile. This area is practically devoid of plant life except

along the coast where fog is a major source of precipitation. Here the *Thelocophala* species was found buried beneath a layer of sand 0.5–2.0 cm thick. Presumably the higher humidity of this microhabitat makes it attractive, despite the fact that the photosynthetically active radiation at a depth of 1 cm is reduced to about 1% of that at the surface.

The prevention of water loss, either by habitat specialisation or by more conventional stomate control, has its cost in terms of production. I. R. Cowan (Australian National University, Canberra) considered water use efficiency in plants in terms of the relative sensitivity of transpiration rate and assimilation rate to changes in stomatal apertures. Changing environmental conditions, such as increasing aridity may place those species at an advantage which have the capacity to vary this ratio, for example facultative crassulacean acid metabolism plants.

Such evidence concerning adaptation in desert plants demonstrates how natural selection in these environments has operated, in Darwin's words, through a "struggle with the elements". Evolution in deserts has apparently been dominated by this struggle rather than by competitive interactions between organisms.

Yet this is not the whole story, for it is among desert shrubs that one occasionally finds regularity of dispersion which betrays an underlying intraspecific competitive interaction which limits seedling recruitment. Such regular patterns have been described both in North America for *Larrea tridentata* (see Fonteyn & Mahall *Nature* **275**, 544; 1978) and in Asia for *Zygophyllum eurypterum*. Interspecific interactions are also known, as was described at the INTECOL meeting by A. Danin (Hebrew University,

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* Held 10–16 September

Jerusalem). Some halophytic species, such as *Reaumuria hirtella* excrete salt from their leaves, thus increasing the salinity of their adjacent soils; other halophytes may have the same effect as a consequence of the shedding of salt-rich leaves. The outcome is suppression of the germination and establishment of glycophytic annuals in their vicinity.

M. M. Caldwell (Utah State University, Logan) provided one example of a situation where one desert shrub had been replaced by another. The competition was between *Ceratoides lanata* (C3 species) and *Atriplex confertifolia* (C4 species) in the Great Basin Desert of North America. Carbon from the roots of *Ceratoides* had $\delta^{13}\text{C}$ values of -22 to -25 , whereas that from *Atriplex* had values of -18 to -19 ,

which is to be expected from C3 and C4 plants respectively. But when soil organic matter carbon was examined for the layers beneath areas now occupied by *Ceratoides* it was found that upper levels had a $\delta^{13}\text{C}$ of -20 to -23 , whereas deeper layers provided values of -15 to -18 . Evidently the C3 species has invaded and replaced *Atriplex* in areas which once it dominated. The C4 system does not always come out on top.

To say that competitive interactions within or between species in deserts are unimportant is thus an oversimplification of the actual state of affairs. It may be that the adaptations which competitive selection has imposed upon desert species are subtle and must be sought at a metabolic level. □

Does Venus have an intrinsic magnetic field?

from C. T. Russell

DECEMBER on Venus promises to be very busy this year. Every 18 months the energy required to launch a probe to Venus reaches a minimum. During this year's energy minimum or, as it is more commonly called, launch opportunity, the USA and the USSR each launched two spacecraft to Venus. The two US spacecraft are scheduled to arrive on 4 December (an orbiter) and on 9 December (a 'bus' carrying one large and three small atmospheric probes). The USSR spacecraft are expected to arrive at Venus in late December and are generally believed to consist each of a lander with a flyby 'bus' rather than an orbiter. Thus in December, we expect that Venus will be visited by six atmospheric probes, two of which will make measurements of the Venusian surface, one bus spacecraft that will burn up in the atmosphere and two that will pass by, and an orbiter which will stay for some time making measurements to altitudes as low as 150 km above the surface.

One of the key questions to be addressed by the orbiter spacecraft is whether Venus possesses an intrinsic planetary field similar to those of the Earth and Mercury. Initial observations with Mariners 2 and 5 and Venera 4 suggested that Venus might not in fact have an intrinsic magnetic field. However, reexamination of some of these earlier data (Russell *Geophys. Res. Lett.*, 3, 125, 1976) and more recent reports of the Venera

9 and 10 orbital magnetic measurements (Dolginov *et al.* *Lunar Science IX*, 256, 1977) have provided evidence in favour of an intrinsic field. The situation is far from clear, and the recent report of plasma measurements by the Venera 9 and 10 wide-angle plasma devices (Verigin *et al.* *Geophys. Res.*, 83, 3721, 1978) does little to resolve this question. There is no doubt from the Venera 9 and 10 data that the obstacle deflecting the flow over the forward hemisphere of the planet is the ionosphere. The authors suggest a stagnation point, or effective obstacle height, of 500 km altitude. In fact it is probably even closer to the planet than this. More recent Russian radio occultation data indicate 260 km to be a more likely obstacle height. Thus any intrinsic planetary field plays only a minor role in deflecting the solar wind. With the solar wind penetrating this close to the planet, charge exchange of the solar wind with the hydrogen geocorona could be an important mechanism in decelerating the solar wind ahead of the planet as suggested by Wallis (Nature, 233, 23, 1971). This mechanism would at a very minimum in the subsolar region weaken the Venus bow shock which slows down and heats the supersonic solar wind before it hits the ionosphere. The authors reject this hypothesis but show no data in the subsolar region. It is still possible that no well-defined shock transition exists in this region.

Verigin *et al.* also report on measurements in the wake region behind Venus. Here they find a plasma

magnetic tail similar in many respects to the terrestrial magnetotail in which a "magnetic field prevents plasma from filling the region behind the obstacle". However, the authors leave open the question as to whether the tail field arises from intrinsic or ionospheric sources.

Verigin *et al.* also compare the Venera data with the Soviet Mars data. In many respects the Mars and Venus plasma measurements are similar, but Mars seems to stand off the solar wind further above the ionosphere than Venus. The authors suggest that this fact indicates that the Martian obstacle to the solar wind is different from the Venusian obstacle, and that the Martian obstacle is indeed an intrinsic field. On the other hand, I find the other evidence for a Martian magnetic field less convincing than for Venus (*Geophys. Res. Lett.*, 5, 81, 1978).

In short the question of the existence of intrinsic planetary magnetic fields for the Earth's two nearest neighbors is still very controversial. We expect that further analysis of the Venera 9 and 10 data and the new data to be acquired in December will resolve this question for Venus. However, there are no plans to my knowledge to probe the Martian magnetic field until at least 1982. At that time the American Galileo spacecraft, as presently scheduled, will whiz by at an altitude of closest approach of 275 km on its way to Jupiter. If I were polled on the eventual outcome of these investigations I would vote yes for a presently operating Venusian magnetic dynamo and no for a Martian dynamo. □

Sex pheromones in bacteria

from J. R. Saunders

THE role of sex pheromones in mating is well established in higher organisms and fungi. In contrast there is scant evidence for such chemical sex attractants in bacteria. In Gram-negative bacteria the formation of aggregates of bacteria during conjugation is well known (see for example, Achtman & Skurray in *Microbial Interactions* (Series B. Receptors and Recognition Vol. 2) 233 (ed. Reissig) Chapman and Hall, London, 1977). The molecular mechanisms involved in genetic transfer during bacterial conjugation have been intensively studied but much less is known of the signalling processes which might initiate contact

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THE sea moths are a small family (Pegasidae) of marine fishes found only in the Indian and western Pacific oceans, from East Africa to Hawaii. They are enigmatic little animals encased in a hard exoskeleton and with relatively small fins except for the pectorals which are close behind the head and which open fanning out in the horizontal plane. It is these large pectoral fins, wing-like at a first glance, which gave them the name of sea moths. In life these fins are 'fluttered' and the fish moves in short bursts over the sea bed—as W. E. Gosline and V. E. Brock (*Handbook of Hawaiian fishes*, 290; 1960) observe of captive fish "this species acts . . . like a not very large butterfly". Curiously for such interesting-looking animals very little seems to be known of their natural history. Most seem to live in shallow water (down to 200 m) on sandy bottoms, presumably relying on cryptic coloration and irregular swimming action to avoid predators, although one species is described as being red in colour with blue eyes!

While their biology and life style are poorly known there is a wealth of discussion, and disagreement, over their systematic position. Recently, T. W. Pietsch (*Copeia* (3) 517; 1978) has reviewed the previous literature and on the basis of his studies of the skeleton of the sea moths has at last provided firm evidence for their classification. As Pietsch points out two main schools of opinion have existed in the past. The earliest discussions placed them with the pipefishes, sea horses, and their relatives, a stance that was general up to the early twentieth century. However, Günther (*An Introduction to the Study of Fishes*, 482–483; 1880) and

Sea moths pinned down

from a Correspondent

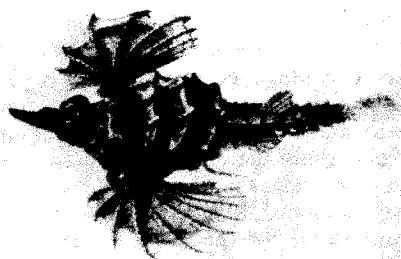


Fig. 1 *Pegasus draconis*. After Day, 1878.

others, impressed by resemblances to certain scorpaeniform fishes (gurnards, scorpion fishes, and especially the poachers) aligned them with this group. Later authors followed this lead, some more tentatively than others, but the general consensus of opinion during the present century has been that they were a distinct order, Pegasiformes, close to but distinct from the scorpaeniform stem.

As a result of an elegant study of the skeleton of *Pegasus*, Pietsch has shown that its upper jaw in particular seems to be unique amongst living fishes although similar, if not identical, to the jaw mechanism of the fossil *Ramphosus* from the Lower Eocene of Monte Bolca and Denmark. There has been general agreement that most of the structures of *Ramphosus* place it amongst the syngnathiform fishes (such as pipefishes). This suggested that *Pegasus* may well be closely allied to the pipefishes and their allies and Pietsch has found a number of significant features which support this view. In addition to the similarities in cranial osteology, the most anterior vertebrae are

elongate which is a feature typical of the syngnathiform fishes; also the gill filaments of *Pegasus* are tufted and lobe-like, not laminate as in most fishes, again a feature of the pipefishes. Pietsch argues convincingly from these and other features that the true relationships of the sea moths lie in fact with the pipefishes and their allies, not with the scorpaeniform fishes.

His further conclusions regarding the classification of these enigmatic little fishes is that they lie intermediate between the two main groups, on the one hand the sticklebacks (themselves a group with a chequered history of classification) and on the other the pipefishes and their allies. All three groups are now united in the order Gasterosteiformes. To allow for the absorption of the new group within the order Pietsch has put forward a new classification in which the sea moths represent a new superfamily. As he points out, this is tentative, as further investigation of several presumed relatives is still required.

It is mildly ironic that these little-known fishes, early examples of which mostly reached Europe as pinned specimens in Chinese insect boxes, have now been placed alongside the sticklebacks and pipefishes which are probably the most intensively studied fishes. The cynic might also point out that in this new classification the sea moths are merely returning to the position they occupied in the mid-nineteenth century. However, on account of Pietsch's careful anatomical comparisons and detailed discussion it seems that this time they will be firmly pinned down—not in insect boxes—but in between the sticklebacks and the pipefishes within the order Gasterosteiformes.

between donor and recipient cells. Although mating signals have been postulated in conjugation little convincing proof has yet been obtained for the presence of specifically 'sexual' attractants. A recent paper from D. Clewell's laboratory (Dunny, Brown & Clewell, *Proc. natn. Acad. Sci. U.S.A.* 75, 3479; 1978) now provides evidence for the existence of a sex pheromone in the Gram-positive bacterium *Streptococcus faecalis*, a normal inhabitant of the gut. Some strains of this organism possess plasmids specifying a number of characteristics including haemolysin production, bacteriocin production and antibiotic resistance. Certain of these plasmids can promote gene transfer by

a process requiring cell-to-cell contact and resembling conjugation in Gram-negative bacteria (Jacob & Hobbs 1974; *J. Bact.* 117, 360; 1974). It has been found that donor and recipient strains of *S. faecalis* form clumps in mating mixtures. Clumping has the effect of bringing donors and recipients into close contact with each other and obviously increases the probability of successful transfer of DNA during conjugation. The formation of these clumps or mating aggregates in *S. faecalis* is apparently induced by an extracellular, heat stable protein of molecular weight between 1,000 and 10,000. This protein, termed a clumping inducing agent (CIA) is produced, perhaps surprisingly, only by recipient (female) cells and not by donors (males). Furthermore, cell-free preparations of CIA will cause donor cells

to adhere even in the absence of recipients. However, CIA is not a physical agglutinating agent but rather a signal (pheromone?) which induces in donors a sequence of events resulting in cellular adhesion. Donor cells must be exposed to CIA for 30 min before clumps are formed. During this time the CIA seems to induce the synthesis of RNA and protein species which are necessary for clumping.

A further interesting property of the *S. faecalis* mating system is that recipients show a reduced ability to elicit CIA once they have acquired a self-transmissible plasmid thereby becoming male. This implies that streptococcal sex factors can regulate CIA either by switching off synthesis of the protein or by inactivating pre-existing molecules of CIA. This would seem to be reasonable in terms of biological econ-

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omy as it would be wasteful for two male strains to mate with each other.

The mode of action of CIA is itself intriguing. It will be necessary to determine the precise nature of the macromolecules induced in donors by CIA and how these could engineer the clumping response. It is possible that CIA may have an additional function in initiating the mobilisation of DNA for transfer in conjugation. The discovery of CIA in *S. faecalis* should stimulate further research to determine whether sex pheromones are a more general phenomenon amongst bacteria. □

Cosmochemistry and evolution

from A. H. Olavesen and
N. C. Wickramasinghe

AN international workshop on Cosmochemistry and the Origins of Life, organised by University College, Cardiff, was recently held at Gregynog Hall, Wales. A broad overview of the conventional ideas of chemical evolution was provided by C. Ponnamperna (University of Maryland), who pointed to numerous laboratory experiments which showed the production of biochemical monomers from suitably energised mixtures of CH_4 , NH_3 and H_2O . Such conditions are usually postulated to have occurred on the primitive Earth. A. W. Schwartz (University of Nijmegen, Netherlands) provided further evidence for the laboratory production of biochemicals from simpler chemicals. A wide range of biochemicals could be synthesised from 70% eutectic mixtures of HCN in water. For instance all the purines were formed spontaneously in such systems, but pyrimidine synthesis required further ultraviolet-catalysed rearrangements. Phosphorylation reactions were envisaged as being derived from active phosphorylating agents arising from the interaction of apatite with certain other products of the HCN reactions. Reactions involving HCN were thought to have occurred most probably over primitive land masses whilst condensations of formaldehyde occurred in the primitive oceans. The two chemical systems are considered to have come together at the land-water interface.

H. Noda (University of Tokyo) discussed the probability of the appear-

ance of a living system from a mixture of present-day biochemical monomers. It was concluded that random molecular combinations could not have led to the emergence of life with any reasonable probability. A. G. Cairns-Smith (University of Glasgow) pointed out that it was unreasonable to expect the most primitive genetic material to involve such a complex organic polymer as a nucleic acid. He considered it more likely that the crystal structure of simpler inorganic materials, such as clay minerals, might have served as some kind of primitive gene. Clay particles might also have served to concentrate organic materials necessary for the origin of life by surface absorption processes.

M. Paecht-Horowitz (Hebrew University of Jerusalem) demonstrated that certain well-directed polypeptide synthesis could be achieved in the presence of some clay minerals. A degree of polymerisation of up to about 24 amino acids was shown to be possible in this way, and the process was thought to be related to the persistent occurrence of blocks of amino acids in present-day proteins. P. Decker (Chemical Institute, Hanover) discussed the role of autocatalytic systems in the early development of biochemistry. Feedback loops in such systems could achieve steady-state concentrations of certain biochemicals without the necessary participation of enzyme catalysts.

J. T. Wimpey (University College, Cardiff) traced the evolution of microbes from anaerobic prokaryotes to eukaryotic structures. Evolutionary pressures are thought to have forced primitive prokaryotes to develop increasingly sophisticated systems for the utilisation and conservation of essential ingredients. He stated that further biochemical evolution was effectively predetermined at the stage at which the first eukaryotic systems emerged.

The question of the site for the origin of life pervaded the entire meeting. Most participants preferred a terrestrial origin, but a radically different proposal by F. Hoyle and N. C. Wickramasinghe (University College, Cardiff) was the subject of much discussion. Hoyle and Wickramasinghe argued that the set of biochemical monomers necessary for the origin of life is produced in mass flows from stars and is abundant in interstellar molecular clouds. Such biochemicals were mopped up into comets when the Solar System formed and high aqueous concentrations of these materials are maintained for long periods in cometary interiors where they propose that life began. Hoyle and Wickramasinghe suggested that comets still continue to shed living systems onto the Earth in the form of bacteria

and viruses. They then discussed an analysis of influenza attack rates in boarding schools (during the 1978 epidemic) which they claim provides evidence against normal explanations for the spread of this virus.

P. M. Solomon (State University of New York, Stony Brook) reviewed astronomical data relating to galactic molecular clouds. Over 40 molecular species have been detected so far and include HCN and formaldehyde which are also the most ubiquitous of the simpler organic molecules in the Galaxy. It is of interest that laboratory synthesis of biochemicals have mainly involved these two cosmically abundant species.

W. M. Irvine (University of Massachusetts) discussed radioastronomical observations of molecules in comets. Molecules detected so far include HCN, CH_3CN , H_2O and several radicals and ions. There are several radio lines in comets which are as yet unidentified. S. Yabushita (Kyoto University, Japan) discussed theories of cometary origin. K. Nandy and D. H. Morgan (Royal Observatory, Edinburgh) reviewed astronomical observations relating to an ultraviolet interstellar absorption feature centred on $\lambda=2200\text{ \AA}$. The most likely contributors to this band are thought to be small graphite spheres of radii less than $200\text{ \AA}$. N. Nakagawa and A. Sakata (University of Electro-Communication, Tokyo) considered the role of organic molecules with conjugated double bonds in producing the $\lambda 2200\text{ \AA}$ band. They also discussed mechanisms for the formation of such molecules by grain surface reactions in interstellar space, and described laboratory experiments which synthesised such molecules as HC_3N , HC_4N , HC_5N and HC_6N from HCN. □

European geophysics

from Geoffrey M. Brown

OF all the branches of science which attempt to operate under omnibus umbrellas, possibly the most nebulous is geophysics. The spectrum of interest extends from what is curiously referred to as 'solid earth' to the outermost extremities of the terrestrial environment. It is evident that in any assembly of geophysicists in conference there will be no uniting theme. The recent meeting of the European Geophysical Society was no exception. If one had to select a single phrase to summarise the conclusions from the wide coverage of subjects it would be to emphasise progress in techniques. Repeatedly there were references to recent advances in measuring techniques and instrumentation, the development of

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new techniques, the application of numerical techniques, and the refinement of analytical techniques.

In the symposium on active plate boundary regions of the North Atlantic, results of the long-range side-scan sonar GLORIA were presented which confirmed the importance of this technique for tectonic studies of such boundaries. New syntheses of the seafloor spreading history of the North Atlantic have been undertaken, and a major land/sea refraction experiment has shown that in the top few tens of kilometres beneath Iceland normal upper mantle velocities are absent. Partial melting is suggested by an increase in Poisson's ratio with depth.

Thanks to major improvements in palaeomagnetic and rock magnetism analysis techniques, increasing detail is now available on the relationship between successive phases of deformation and metamorphism, and on estimates of parameters such as age and temperature. The general character of the palaeomagnetic record is now so well established that claims to discover new 'anomalies', whether in general palaeomagnetism or magnetostratigraphy, must pass rigorous standards of analysis before they can be considered seriously.

In fluid dynamics, which spans the atmosphere, oceans, mantle and core, much progress has been made by combining field observations, laboratory experiments and numerical analyses. For example, in thermal convection some of the patterns (rolls, hexagons, knots, skewed varicose instabilities) identified in the laboratory, are now recognised (though not yet fully understood) in clouds in the atmosphere and even in small boulder formations in Arctic regions. The effects of condensation on mountain lee waves can now be modelled, whilst on a larger scale this is evidence that the global circulation may be quantised into a number of stable and unstable states, transition being effected by small-scale perturbations. Transition probability is a major problem.

'External geophysicists' were served by three symposia. In one, concerned with the aeronomy and dynamics of the lower ionosphere, the main message was the importance in the height region 70–90 km, of the influence of the neutral atmosphere on the ionised atmosphere. The mechanisms which control this ion-neutral coupling are being intensively studied. In general, the rate of production of ionisation is largely determined by the transport of atmospheric minor constituents, such as nitric oxide, while the rate of loss

of ionisation is strongly dependent on the extent of the water cluster-ion regime which, in its turn, is controlled by the temperature of the atmosphere. Knowledge has significantly advanced through the extension and improvement of both ground-based and rocket techniques. Particular attention was given to results from the Western Europe Winter Anomaly Campaign of 1975–76, which have demonstrated that anomalously high radio wave absorption in winter arises from both increased production and decreased loss of ionisation in the D region. Greatly improved digital telemetry in rocket experiments reveals a fine structure in both the ionisation and neutral gas density in the lower ionosphere. As another example of progress in techniques VHF radar using very short pulses integrated over a second has been used to detect very thin vertically moving layers in the height range 60–75 km.

The other two atmospheric symposia were concerned with different aspects of the acceleration of charged particles in the Earth's environment. The mechanisms which impart the necessary energy to the particles responsible for the aurora have long been elusive. In the symposium on auroral acceleration processes, experimental auroral data covering a range of parameters were compared with theoretical predictions, computer simulations and laboratory studies of plasma acceleration mechanisms. The role of electric fields parallel to magnetic field lines within an Earth's radius is being actively studied. The injection of tracer beams of electrons and ions promises to be an important new technique in the experimental measurement of such fields. Valuable new insights are expected to come from more detailed experimental study of particle distributions, there is need for clarification of the association between accelerated particles and plasma waves. The consensus was that acceleration by quasi-static electric fields is probably the most viable model to date, although it is clear that no single theory is currently capable of accounting for all observations.

In the symposium on the bow shock, consideration was given to the acceleration of charged particles in space in the collisionless region ahead of the magnetosphere where wave-particle interactions transform the cool, supersonic solar wind in the interplanetary medium into hotter, lower speed plasma in the magnetosheath. Very recent measurements aboard the first two ISEE (International Sun-Earth Explorer) satellites, only a few hundred kilometres apart, were presented which enable temporal variations to be resolved from spatial

variations for the first time. These show the presence of ions, sometimes suggestive of beams with rather well-defined angular distributions and with energies up to a few keV, believed to be accelerated by wave-particle interactions at the bow shock. They also show that the thickness of the shock may vary by a considerable factor. The detailed ISEE observations now available in space will remove some of the ambiguities of single spacecraft measurements and should lead to renewed theoretical efforts to understand the intricate physics of collisionless shocks in plasmas.

The EGS is a young society which seeks to bring together geophysicists of all types through its annual meetings. Already detailed plans are laid for the sixth meeting in September 1979 in Vienna. □

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Liposomes in the therapy of lysosomal storage diseases

from Gregory Gregoriadis

THE first lysosomal storage disease was identified by Fers in 1963, who found that deficiency in lysosomal X-glucosidase resulting in glycogen accumulation was the underlying cause of the clinical manifestations of Pompe's disease (glycogen storage disease type II). Other lysosomal storage diseases (LSDs) were subsequently discovered which involve the total or partial absence of various hydrolytic enzymes. Initial attempts at enzyme replacement therapy by the direct administration of the missing enzyme isolated from foreign and human sources were disappointing (reviewed by Desnick *et al. Physiol Rev* 56, 57, 1976). Patients set up immunological reactions to the foreign enzymes and some enzymes even fail to enter afflicted cells. Enzyme is also wasted through premature inactivation or loss to irrelevant areas (see *Enzyme Therapy in Lysosomal Storage Diseases* (eds Tager, Hooghwinkel & Daems) North Holland Publishing Company, Amsterdam, Oxford, 1974).

Some years ago liposomes were proposed as suitable enzyme carriers (for a recent review see Finkelstein & Weissmann *J Lip. Res.* 19, 289; 1978).

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*Held at the Université Louis Pasteur, 29 August–1 September.

Biochemical and morphological observations showed that, *in vivo*, liposomes could transport agents into the lysosomes of cells (mainly those of the reticuloendothelial system and the hepatic parenchymal cells) by endocytosis. Liposomes protect their enzyme contents from circulating antibodies and prevent them from attacking normal blood components. Work with model lysosomal storage 'diseases' suggested that enzymes transported in this way into lysosomes could degrade stored materials, and recently fibroblasts from a cystinotic patient were partially depleted of lysosomal cystine after exposure to liposome-entrapped cysteamine.

Reynolds *et al.* (page 754, this issue of *Nature*) now present a method for evaluating the liposome approach to enzyme replacement therapy in a naturally occurring LSD, namely feline GM₁ gangliosidosis, which is characterised by a deficiency in β -galactosidase. Skin fibroblasts from cats with GM₁ gangliosidosis were initially pulse-labelled with radioactive galactose (galactose-labelled glycopeptides in the lysosomes of diseased cells are not catabolised over a period of days). Exposure of galactose-labelled cells to liposomes containing β -galactosidase from feline liver is followed by the catabolism of the radiolabelled glycopeptides as a result of the concomitant uptake of the enzyme, apparently through endocytosis of its liposomal carrier. In this and other cases free enzyme fails to enter the cells.

Clearly this is an attractive way to measure the efficacy of a given liposomal enzyme preparation within target cells and could be easily applied in a variety of LSDs. However, degradation of pulse-labelled substrate, obviously concerning newly synthesised materials, may not be characteristic of enzyme action on all accumulated substrate in all intracellular areas. Indeed, it is possible that lysosomes which are either aged or excessively loaded may not be able to fuse with the endocytic vacuoles transporting the liposomes or with young secondary lysosomes which have already incorporated the enzyme. In addition, much of the substrate may be in a physical state resistant to enzyme activity. Therefore, experiments are now needed to show that what Reynolds *et al.* achieved with pulse-labelled fibroblasts can also be achieved with cells at the centre of a given LSD (for example, fixed macrophages from adult Gaucher's disease patients storing glucocerebroside, cardiac or skeletal muscle cells from glycogen storage disease type II patients) by measuring total stored material and examining cellular morphology and function before and after exposure to enzymes.

Extrapolation of results from *in vitro*

experiments to the overall efficacy of the system *in vivo* could, however, be meaningless. In dealing with animals one should consider difficulties related to the journey of the carrier to areas in need of treatment. Liposomes must retain the enzyme while in the circulation and must therefore be designed appropriately. In several LSDs involving tissues other than liver and spleen, the carrier must cross membranes to reach and enter diseased cells (entrance into nerve cells seems improbable at present) and control of its size and surface characteristics will be needed (for a review see Gregoriadis *Nature* **265**, 407; 1977). Reduction in size will also ensure less interference by the liver and spleen, although the chances are that these tissues will themselves be enzyme deficient and participation in uptake is unlikely to be detrimental. Possible toxicity of liposomal lipid components and increased immunogenicity of administered enzymes promoted by the carrier must also be taken into account (discussed by various contributors, *Ann. N.Y. Acad. Sci.* **343**: 1978).

Assuming that enzymes reach target cells and degrade much of the stored material, will this then lead to the organ functioning normally? In adult Gaucher's disease for instance, liver and spleen grow enormously with con-

comitant development of fibrous tissue and other secondary morphological as well as metabolic disturbances, and in several lipidoses nerve cells are probably irreversibly damaged. Our experience (Belchetz *et al.*, *Lancet* **i**, 116; 1977) with an adult Gaucher's disease patient being treated for over 30 months with liposomes containing glucocerebroside: β -glucosidase isolated from human placenta, provides evidence that the enzyme is acting in the liver, as the transient pain in that area after each injection is proportional to the amount of enzyme given. Significantly, the patient's health has stopped deteriorating since treatment began and there is some reduction in abdominal pain due to liver pressure as well as some improvement of reticuloendothelial system function. In the only other case of LSD treatment with liposomes some reduction in liver size was also reported. Unfortunately, however, the patient (an infant with glycolipidosis type II injected with fungal amyloglucosidase) died before any valid conclusions could be drawn (Tyrrell *et al. Brit. Med. J.* **2**, 88; 1976). A major difficulty in the follow-up of LSD patients is our inability to measure progress in serial biopsies as there are gross differences in the substrate content and morphology of various parts of the tissue. □

Radiation research in Scandinavia

from R. B. Cundall

A RECENT conference* in Denmark covered most of the topical and debatable aspects of radiation science, in addition to what now appear to be well established and steadily progressing areas, such as electron trapping, excited states, and free radical reactions. The conference was attended by delegates mainly from Scandinavia, and served particularly well in the attention which was directed to controversial and timely topics. These were the disposal of nuclear waste, industrial applications of high energy radiation, and radiation effects on DNA.

Legislation recently introduced in Sweden on the disposal of spent fuel and wastes from nuclear power generation has served to concentrate much effort and thought on this subject. Papers by F. Lofveberg (Nuclear Fuel Safety Project, Sweden), A. Lund (Nykoping, Sweden), J. A. C. Marples (AERE, Harwell, UK) drew attention to the nature and properties of vitrified waste and the thought-provoking prob-

lems of storage facilities which may be used for periods of more than 10⁶ years. The possibilities of leaching, temperature effects and washing which could be significant have been examined but the results indicate that the radiation and physical stability of glass waste is of no concern. Presentations on transport of radioactive isotopes through different types of terrain also provided reassurance. This type of work is important for the safe exploitation of nuclear power, especially since the time scale of possible hazards is outside previous human experience and could involve larger quantities of dangerous material than is generally appreciated, although so far the radiation experts' findings are encouraging.

Opposing views are even more firmly established concerning possible industrial applications of ionising radiation to chemical synthesis and modification of physical properties, especially those of polymers. J. Silverman (University of Maryland), a well-known exponent of radiation technology, spoke with enthusiasm and probably surprised the delegates with economic statistics showing possible annual turnover

*6th Conference of the Nordic Society for Radiation Research and Technology, Hirtshals, Denmark, 28-31 August 1978.

"SUDDENLY, pear blossom on every tree/As though the wind of Spring came upon us last night." With these words so welcome to a people that has just passed a long and dreary Winter in science (see *Nature*, 274, 834; 1978), a new Chinese journal, *Ziran Zazhi*, greets the world.

Ziran Zazhi means simply 'Nature Magazine'. It is a monthly of about 60 pages and its first issue came out in May. Its aims are: to get better acquainted with Nature, to remould Nature and to exact greater freedom from Nature. Its editorial policy is to "let scientific truth develop and grow through contention". It covers both physical and biological sciences and is very much like this Journal in having as its main content Articles, Research Letters and Nature Information. In addition, it has two more popular sections, Explorations in Nature and Riddles in Nature.

The Research Letters at the moment account for only 10% of the pages, but they are given pride of the place. Compared with the Letters of this Journal, they are somewhat shorter, just as technical, and cover as wide a spectrum, except perhaps for a slight bias toward particle physics and systems analysis in the issues so far published. Arguably the most original Letter so far is a report of some experimental results on the physical basis of the traditional *yunqi* therapy—the curing of disorders through the passing of *qi* (pneuma) from doctor to patient without bodily contact. Though not as well-known as acupuncture, this form of therapy has its believers and followers among

Nature: Chinese style

from T. Kiang

the Chinese people. It goes something like this: the doctor does a sort of breathing exercise; a few minutes later, he has a feeling of congestion (stage 1) which may last a couple of minutes; he then has a feeling of release (stage 2) lasting another couple of minutes. The traditional 'theory' is that, during stage 2, the doctor's *qi* is emitted through certain specific points called *xuwei* and enters the patient's body through some other specific *xuwei*, effecting the desired cure. What the experimenters found was that, during stage 2 but not during stage 1, the far infrared radiation at 1.2 cm from the active *xuwei* of the doctor showed a slow (down to 0.05 Hz) modulation of large amplitudes (up to 80%). They also found abnormalities in body electricity near the *xuwei*, during both stages. Furthermore, from chemical analysis of tissues from a number of corpses, they found that the tissues at the *xuwei* points had a marked excess of nickel. These findings have all the hallmarks of pioneer work and their preliminary character makes the Research Letter their perfect vehicle.

The Articles form the mainstay of ZZ. Each covers in depth a certain topic—Mössbauer spectrometry, the red tide and laser chemistry have been covered amongst others—and is written by an acknowledged expert in the field. In regard to the degree of

technicality, the Articles of ZZ resemble more their counterparts in *Endeavour* than those in this Journal.

Nature Information consists of extracts of articles from other current scientific periodicals, notably, *New Scientist*, *Science News*, *Science* and *Physics Today*. Here, as with Articles and Research Letters, astronomy and astrophysics have their due share, but these are even more prominent in the Explorations in Nature section. In one form or another, readers have been informed of recent findings made in China and abroad on the origin of the Solar System, black holes, gamma-ray bursts, neutron stars, rings of Uranus and quasi-stellar objects that are at the same time double radio sources. For many of the Chinese investigations, the full-length papers are now available in English in the translation journal *Chinese Astronomy*.

The Riddles in Nature which have appeared so far have dealt with the life-span of single cells, magnetic monopoles and the μ -particles I, as a professional astronomer, found the first most intriguing—which the editors of the multidisciplinary *Ziran Zazhi* may feel gratified to know.

The appearance of *Ziran Zazhi* is a heartening event. Just how heartening can perhaps be appreciated only by the Chinese scientific community. It is one of the signs that the country has begun her 'New Long March' towards modernisation through science and technology. □

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reaching billions of dollars in the USA alone. Two hundred electron accelerators and 60 industrial ^{60}Co units are already in use throughout the world. It is clear that academic workers in the radiation science area are ill informed on the industrial possibilities; for example, well publicised applications to the synthesis of detergents seem to have failed whereas the radiation-induced crosslinking of rubber for tyres has apparently been very successfully applied by the Goodyear Company in the USA. Discussion revealed caution and open scepticism from many delegates about the current state of such technology, although specific and restricted areas of application, sterilisation for example, are undoubtedly extremely useful. Industrial application depends crucially on detailed investiga-

tions and these were exemplified by papers on polyethylene/SiO₂ composites, polystyrene sterilisation, and electrical insulation. J. Rabani's (Hebrew University, Jerusalem) interesting paper on solar energy exploitation was more of hope and problem definition than achievement. Photoelectron transfer effects which are probable mechanisms for achieving the formation of H₂ and O₂ from solar irradiation of water are likely to be enhanced by polyelectrolytes, ion exchangers and micelles. The use of polyelectrolytes seems to offer many significant advantages since they inhibit back electron transfer reactions and help fulfil the requirements of energy storage.

The interest in cellular and sub-cellular radiobiology is largely centred on the effects of radiation on DNA, and the effect of oxygen was the main theme of the presentation. The difficulties of experimentation in this clinically important field were highlighted by the

paper of B. M. Cullen and J. W. Boag (Hammersmith Hospital & Institute of Cancer Research, Sutton, UK) which showed how errors of technique can provide misleading results. Photoelectrons formed in higher yields when argon was used instead of nitrogen as deoxygenating atmosphere caused reductions in cell survival.

The still incomplete knowledge on reactions of the biochemically significant O₂⁻/HO₂ system was also examined. The paper by G. Czapski (Hebrew University, Jerusalem) showed the increasing involvement of physical chemists in problems of radiation biology. The interdisciplinary approach is proving seminal, the previously accepted so-called Haber-Weiss reaction is discounted but an alternative explanation remains to be found.

No enzyme has attracted the radiation scientist more than superoxide dismutase. The reasons are its selectivity towards the O₂⁻/HO₂ system and

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its convenient properties for study by pulse radiolysis. E Lengfelder (Strahlen-biologisches Institut der Universität, Munich) showed how several synthetic metal-containing complexes also display marked superoxide dismutase activity.

As appropriate for a meeting in Scandinavia significant 'state of the art' papers on DNA by Graslund and G. Ahnström (Wallenberg Laboratory, Stockholm) and positronium (O. Mogensén, Riso Laboratory, Denmark) were presented. The effect of radiation on DNA has been and remains the most important aspect of radiobiology and it is essential that effects of individual free radical reactions, strand breakage, chemical modification and biological effects are examined and correlated. Ahnström emphasised that effects on repair processes could be crucial to radiosensitivity. Radiation damage to DNA prevents enzymic cleavage of oligonucleotides, and suggests a new method for studying radiation effects in DNA (D Schulte-Frohlinde and C von Sonntag, Max-Planck-Institute, Mulheim).

The successful application of resonance Raman spectroscopy to pulse radiolysis was a notable achievement of the Riso group (R Wilbrandt, N H. Jensen and P. Pagsberg) and details of the structure of (SCN)₂⁻ have been determined by its use. The powerful technique of pulse radiolysis is considerably improved by transient detection methods which allow better structural identification. □



A hundred years ago

In a lately-issued number of the *Proceedings* of the American Philosophical Society, Prof Cope has given an account of the collection of fishes made by Prof Orton at various points on the head-waters of the Amazon. Species of the genera *Belone* and *Tetrodon*—characteristically marine forms—are here recorded as having been obtained in these districts 2,500 miles from the mouth of the river—thus showing how far marine animals will penetrate into, and become ultimately acclimatised in, fresh waters.

The existence of the true heath plant in North America was for a long time considered very doubtful, and its detection in New England some years ago is a matter of much interest. The published localities hitherto are Newfoundland, Nova Scotia, and Massachusetts, but, according to the *Bulletin* of the Torrey Botanical Club, Dr Hexamer, of Newcastle, has lately found a few plants of it near Egg Harbour, New Jersey.

From *Nature* 18, 24 October, 682; 1878.

Producing more food

from W. S. Wilson

THE inexorable increase in the world's population without a commensurate increase in food supplies has prompted a re-examination of production methods, post-harvest care of produce and even the types of food consumed. These considerations were well to the fore at the XXth International Horticultural Congress*. The wide range of scientific disciplines involved focused on fruit, vegetables, ornamental and amenity plants and post-harvest horticulture. The last was a new feature of these 4-yearly gatherings and reflects the growing concern for quality produce in developed countries and the need to overcome scarcity in many developing countries, including India where per capita consumption of vegetables is only one-third of that recommended by dieticians.

Nowadays vegetables and fruits are survival foods in some countries and health-giving foods in all others. Vegetables provide more carbohydrate, vitamins and minerals per unit area of land than other type of crop. Some, such as the soya bean, are an inexpensive source of protein. A spectacular way of intensifying vegetable and fruit production in some countries was given by E C. Quisumbing (University of the Philippines) when he described the growth of coconuts under which were growing bananas, under which were grown pineapples beneath which were grown sweet potatoes, all on the same piece of land at the same time. This arrangement resulted in quadrupling the potential yield of the land and increasing the income of the small farmers.

Apart from management aids to increasing production and improvement of produce quality, advances in fundamental knowledge and technological applications which make handling and mechanical harvesting easier, were reported. In the temperate fruit section, the emphasis was on the adjustment of tree density by breeding or hormonal treatment to render the fruit easier to harvest mechanically. This trend is particularly advanced in Australia and New Zealand, countries with small labour forces. Plant breeders have introduced dwarfing characteristics enabling more trees to be grown more productively per hectare. Another method for increasing yield of apple cultivars, described by A. I. Campbell (Long Ashton Research Station, Bristol) was the exposure of dormant scions to gamma irradiation under water to form stable mutants. Physiologists have been able to control growth characteristics

by hormonal regulation. Thus ethylene can be used to increase flowering, offset biennial bearing, prevent pre-harvest fruit drop and advance or delay fruit maturity.

The most important advances in vegetables have also been related to systems methods of production. W. L. Sims (University of California, Davis) outlined a sophisticated system for tomato production involving an integrated breeding programme, uniformly flat seedbeds, simultaneous ripening and a single harvest operation. This system is appropriate to California but other systems applicable to other regions can be devised. For less ambitious objectives crop increases can be achieved by deeper loosening of non-compacted soil or by hormonal treatment of crops to achieve greater lateral proliferation of roots to explore compacted soil. Allied with these measures is a rapid method of forecasting fertiliser requirement such as that described by D J Greenwood (National Vegetable Research Station, Wellesbourne, Warwick). This involves assessing inorganic nutrient ions in plant sap with test strips and in the soil filtrate with reagents prepared in tablet form. A notable achievement in vegetable breeding is the production of high protein potatoes (over 10% protein) for use as animal feed in Australia.

The main concern of the section on ornamental plants was to devise the best way of educating the community to appreciate plants, gardens and landscape. For over 300 years botanic gardens have been preoccupied with taxonomy and amenity horticulture. In the future, these institutions and horticultural societies should be more concerned to inject greater diversity into horticulture by, for example, introducing plants from the southern hemisphere into northern countries.

In the post-harvest section a paper by M. A. Perring (East Malling Research Station) on mineral and physiological disorder in apples explained how the physical and chemical composition of apples is influenced by soil type and management, environment and nutrition. In this connection, bitter pit in apples caused by calcium deficiency can be alleviated by dipping the fruits in a calcium chloride solution. Preservation of condition in easily-perishable vegetables can be achieved by covering the crop with low cost polyurethane foam in cool stores.

Australia provided an excellent venue for the Congress, offering a wide range of wild flora alongside a diverse horticulture in which science and technology are complementary. □

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* Held on 15-23 August in Sydney and sponsored by the International Society for Horticultural Science.

review article

The biochemistry of complement

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Current biochemical studies of the complement system are illustrated by description of the activation of complement by the classical pathway after interaction with antibody aggregates. This is described in terms of the structures of the components involved, their assembly and the mechanism of activation.

IN the blood of vertebrates several activation systems have been recognised which are essential for the survival of the animal. These include blood clotting, clot lysis, the renin-angiotensin and kinin systems and complement; all are dependent, at least in part, on the sequential activation of a series of proteolytic zymogens¹.

Complement is the most complex of these systems and it is of interest for several reasons. First, as recognised 80 yr ago², the complement system is responsible for the destruction and elimination of foreign material from the body, notably bacteria and viruses. This usually occurs after the interaction of antibodies with the foreign organisms or substances, and complement is thus the effector mechanism in the immune defence against infection. Complement may also be activated by complex polysaccharides³ such as yeast or bacterial cell walls before reaction with antibody and hence it can form an immediate defence against infection before the development of immunity⁴. Second, receptors for activated complement proteins, such as the third component C3 are present on lymphocytes, macrophages and some other cells⁵⁻⁷, and there is evidence, as yet incomplete, that the lymphocyte C3 receptors may play an important part in the interaction of the different classes of lymphocytes or their precursor cells⁸. The interaction of lymphocytes such as the B and T cells is necessary for the occurrence of the immune response and it is possible that complement may play a central part in the immune response as well as being the major effector mechanism. Third, it has been found that several complement proteins are coded for by structural genes which map into the major histocompatibility locus in man and in mice⁹. The biological significance of these findings is uncertain, but it has been suggested¹⁰ that it may imply that some complement proteins have a role as surface proteins, as all the other proteins coded for by this gene complex apparently do.

Not surprisingly, therefore, interest in the complement system has increased greatly, and the progress in isolating and characterising the various components has led to attempts to determine their structure and the biochemical mechanisms of the activation system. It is highly complex and includes about 20 serum proteins, most of which are part of the activation sequence, but control proteins are also included; these inhibit different steps or inactivate some of the components. The biological importance of the system has been emphasised by the finding that it is probably present in invertebrates and has been conserved throughout evolution¹¹ to such an extent that some components are interchangeable between species as distant as frog and guinea pig¹². The complexity of the system may be due to the multiple role of some components as suggested above, but also,

as with the other activation systems of the blood, it is important that they are activated rapidly when needed but never activated accidentally. A cascade system with each step able to be inhibited offers a system of rapid activation coupled to a series of fail-safe controls.

There are several features of complement which are most unusual. For example, C1q, the component which interacts with aggregated antibody, has a unique structure which is half globular and half collagen-like¹³. Also, several of the proteases involved in the complement system are formed only after activation of the protein possessing the catalytic site and association of this protein with one or more other proteins which thus determine the specificity of the activated enzymatic site^{14,15}. No attempt will be made to review the whole complement system, but only a few of the more uncommon features will be described, of the structure and activation of the early components of complement after interaction with aggregated antibody.

Activation of the complement system

Activation of the complement system can take place by two distinct pathways, the classical and the alternative pathways: here we shall concentrate on the classical pathway. Both pathways have the same terminal components, C5 to C9, which are directly responsible for membrane damage. The difference between them lies in their early-acting components and thus in the manner by which they are activated by immune complexes or other factors. The sequence of events in the activation of the early components of the classical pathway is shown in Fig. 1. When C1 is bound to antibody aggregates it becomes an active protease, C1_s, and catalyses the activation of C4 and C2 which together form another protease C4₂ (ref. 14). This enzyme is also known as C3 convertase because it activates C3, some of

Fig. 1 Activation of the classical pathway by antibody-antigen (Ab-Ag) aggregates.

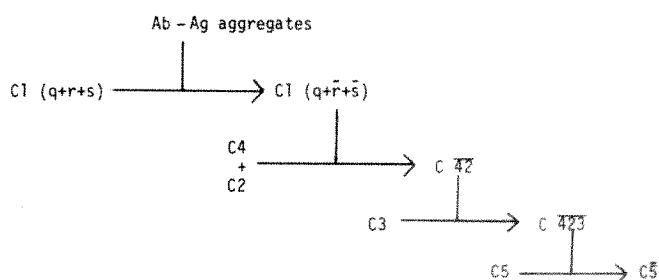


Table 1 Early acting components of the classical pathway

Component	Molecular weights of				Enzymatic site present in activated form	Approximate serum concentration (mg l ⁻¹)
	Intact molecule	Major unactivated form Chains*	Intact molecule	Activated form Chains*		
C1q	410,000†	18 6A 24,000 6B 23,000 6C 22,000		Unchanged	—	150
C1r	83,000‡	1	83,000	56,000 27,000	+	50
C1s	83,000	1	83,000	56,000 27,000	+	50
C2	110,000	1	110,000	70,000 30,000	+	15
C3	180,000	2 α 105,000 β 75,000	170,000	α' 95,000 β 75,000	—	1,200
C4	206,000	3 α 93,000 β 78,000 γ 33,000	200,000	α' 87,000 β 78,000 γ 33,000	—	400
C5	180,000	2 α 105,000 β 75,000	170,000	α' 95,000 β 75,000	—	80

* Chains obtained after reduction and alkylation of disulphide bonds.

† C1q is composed of nine subunits, each subunit consisting of two disulphide-linked chains (see text and ref. 13).

‡ C1r is present as a dimer of two identical, noncovalently linked chains in serum.

which associates with C3 convertase to form C423; which in turn activates C5. Some properties of components C1 to C5 are shown in Table 1. In the alternative pathway of activation, which can be started when polysaccharides are added to serum without interaction with antibody, other components lead to the formation of a different form of both the C3 and C5 convertase enzymes¹⁶. The cleavage of C5 is considered to be the last enzymatic step in the activation of complement by either pathway. The final event is the formation of the C5b6789 complex, apparently by self assembly¹⁷ in the process of which part of the complex becomes inserted into cell membranes^{18,19}, thus causing the membrane lesions seen in electron microscopy studies^{20,21}.

Structure and properties of the first component of complement

C1 occurs in serum as a weak association of C1q with a tetrameric complex of C1r₂-Ca²⁺-C1s₂ which is formed in the

presence of Ca²⁺ (refs 22, 23). Interaction of C1 with aggregated antibody is through C1q (ref. 24) which binds in the absence of C1r₂-Ca²⁺-C1s₂ to the second constant domain (N terminal half of the Fc) of IgG (refs 25–30). The C1r₂-C1s₂ complex binds to C1q on the antibody through C1r, as C1r alone, but not C1s, interacts with antibody bound C1q (refs 31, 32).

C1q is a 400,000 molecular weight protein^{33–35} formed from 18 peptide chains 6A, 6B and 6C, each of about 200 amino acids and containing 12, 8 and 4% CHO respectively³⁵. Amino acid sequence analysis showed that these three types of chain are very similar and each has about 80 residues of typical collagen-like sequences starting close to the N terminal end^{36,37}. The N terminal sequence of the B chain (Fig. 2) between residues 6 and 89 has the repeating triplet Gly-X-Y, where the residue in the Y position is often hydroxyproline or hydroxylysine. The A and C chains have similar collagen-like sequences between residues 9 and 89 and 3 and 89, respectively. (Numbering of residues is based on B chain sequence, Fig. 2.) The repeating triplet sequences in each chain are, however, not quite continuous. In chain B, alanine not glycine occurs at position 9; in chain A

Fig. 2 N-terminal amino acid sequence of the chain of human C1q. The sequences are given in the single letter code: A, Ala; C, Cys; D, Asp; E, Glu; F, Phe; G, Gly; H, His; I, Ile; K, Lys; L, Leu; M, Met; N, Asn; P, Pro; Q, Gln; R, Arg; S, Ser; T, Thr; V, Val. The numbering is based on the B-chain sequence. A gap has been made between positions 38 and 39 in the B and C chain sequences to allow for the 'extra' threonine in the A-chain sequence. A triplet gap has been made between residues 42–44 in the A and C chain sequences, as this seems to give the optimum alignment for maximum homology. The sequences shown are from refs 36 and 37 except for residues C41 to C97 which are unpublished data (K.B.M.R.). Each chain is approximately 200 residues long. * Denotes a hydroxylated residue.

	1	10	20	30	40	50	60
A-chain	E D L C R A P D G K K G E A G R P G R R G R F G L K G E Q G E P G A P G I R T G I Q - - - G L K G D Q G E P G P S G N P G						
B-chain	E L S C T G P P A I P G I P G I P G T P G P D G Q P G T P G I K G E K G L P - G L A G D H G E F G E K G D P G I P G D P G						
C-chain	N T G C Y G I P G M P G L P G A P G K D G Y D G L P G P P G E P G I P A I K - G I R - - - G P P G Q K G E P G L P G H K G						
	61	70	80	90			
A-chain	K V G Y P G P S G P L G A R G I K G I K G T P G S P G M I K D Q P R P A F						
B-chain	K V Q P K G P M G P K G P P G A P G A P G P K G E S G D Y K A T Q K I A F						
C-chain	K D G P N G P P G M P G V P G P M G I P G E P G E G R Y K Q K F Q S V F						

threonine is inserted in between positions 38 and 39 and alanine replaces glycine at position 36 in the C chain. However, with the exception of these small changes at the beginning and in the middle, there is a continuous sequence in all three chains of which the similarity to collagen is increased by the presence of glucosylgalactosyl disaccharide substitutions on the hydroxylysine residues^{33,38}. The sequences of the remaining 110 noncollagenous residues in each chain, which are known, are very similar and each has one intrachain disulphide bond. Collagen fibrils are formed from three peptide chains each in a minor helix, which associates noncovalently to form a major helix with well characterised dimensions³⁹. This makes it possible that the 18 chains of C1q are associated through their collagen-like sections to give a hexameric structure of six fibrils with six globular heads formed from the C-terminal halves of each chain. Such an arrangement would be consistent with the remarkable structure of C1q as seen in electron microscopy, showing each molecule to resemble a bunch of six tulips with the lower half of the stems held together and branching in the upper half to give six separate stems each of which is joined to a globular head⁴⁰⁻⁴².

C1q, when reduced and electrophoresed on polyacrylamide gels containing sodium dodecyl sulphate, shows the three expected polypeptide chains A, B and C in equal amounts which, though of similar size (23,000 MW), separate probably because of their differing carbohydrate content³⁵. However, if electrophoresed before reduction, two subunits can be seen which again, though of similar molecular weight (46,000-48,000), separate clearly on the acrylamide gels. The apparently larger subunit contains the A and B chains and the apparently smaller subunit only the C chain^{13,34}. That is, they correspond to an A-B dimer and a C-C dimer and yields show that there must be six AB and three CC dimers¹³. The association of these nine subunits to yield the hexameric structure seen in the electron-microscopy studies may be achieved in the way described in Fig. 3. A triple helix could be formed between the collagen region of one of a pair of C chains and the collagen regions of an AB dimer; in this manner three pairs of triple helices would be formed. Then the association of these three pairs, again non-covalently, would give the complete hexameric structure, each of the globular heads being composed of the C-terminal 110 amino acids of an A, a B and a C chain (Fig. 3)¹³. The breaks in the Gly-X-Y sequence mentioned above and occurring at positions 39 and 36 in the A and C chains, respectively, could account for the bend in the collagen-like fibrils seen midway along the stems of the molecule, as a true triple helix cannot be formed in this area of the molecule because of the prerequisite for glycine as every third residue³⁹. Measurement of the dimensions of the molecule on the electron microscope picture and calculation from the known lengths of the Gly-X-Y triplet sequence in collagen agree well¹³. It seems likely, therefore, that the postulated structure is correct.

C1r and C1s are very similar proteins, each a single polypeptide chain of about 83,000 molecular weight differing only in that C1r dimerises whereas C1s remains as a monomer in solution irrespective of the presence of Ca^{2+} (refs 43-45). Together they form a tetrameric complex in the presence of Ca^{2+} , as in serum. On activation, they are both hydrolysed to give two disulphide-linked peptide chains of 56,000 and 27,000 molecular weight, but they remain in a tetrameric complex. C1r and C1s are very similar in amino acid content and also in sequence as far as this is known, and it is considered likely that one arose from the other by gene duplication. Both activated subcomponents are typical serum esterase proteases inhibited by diisopropyl fluorophosphate⁴⁶⁻⁴⁸ and both have a characteristic N-terminal sequence present in the 27,000 molecular weight chain (which is the chain containing the catalytic site), showing obvious homology with other serine esterase proteins⁴⁵. C1r and C1s differ markedly in enzyme specificity, however, and C1s is the only known substrate for C1r. C1s, but not C1r, will activate C2 and C4, and C1s will also hydrolyse several peptide substrates, showing a plasmin-like specificity⁴⁵.

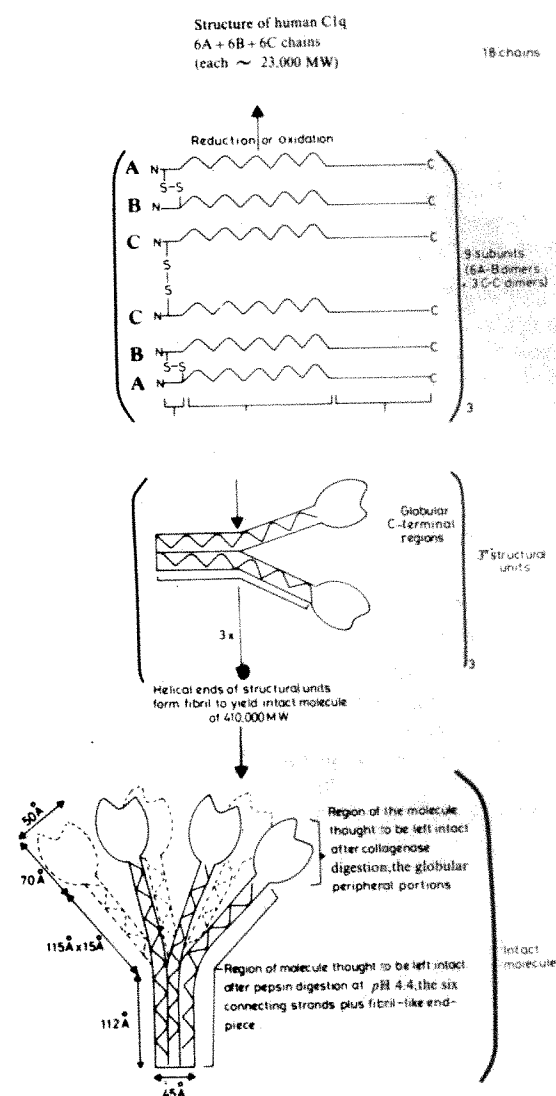


Fig. 3 Proposed peptide chain structure of C1q. The dimensions given are averages of those of Shelton *et al.*⁴¹ as estimated from electron microscopy studies. ~, Proposed triple helix sections, that is, collagen-like regions of molecule. The following comparison can be made. Length of collagen-like fibre + fibril-like endpiece = $115 + 112 = 227 \text{ Å}$. Length of triple helix proposed from sequence studies = $80 \times 2.9 = 232 \text{ Å}$.

Structure and properties of components C2 to C5

C4, a major complement component (400 mg per l serum) has three disulphide-linked peptide chains of about 90,000, 75,000 and 30,000 (refs 49, 50), although recent evidence suggests that it may be synthesised as a single peptide chain of 200,000 molecular weight⁵¹. Some of this precursor molecule, which is very similar to C4 as judged by antigenic specificity, but which has no haemolytic activity, also seems to be present in serum⁵². The Ss protein identified first as a polymorphic protein in mouse serum which is coded for by a gene in the major histocompatibility complex⁵³, has now been identified as C4 (refs 54-57). Human C4 has also been shown to be coded for by a gene which maps into this complex on chromosome 6 (ref. 58).

C2 has been one of the most difficult components to isolate, as it is present at only 15-20 mg per l of serum and is exceptionally vulnerable to proteolysis during isolation. Isolation has now been achieved^{59,60}, and in confirmation of earlier work^{61,62} it was found that C2 is a single-chain protein of about 100,000 molecular weight which is split on activation to two chains, not disulphide-linked, of 70,000 and 30,000. The activated protein may be a protease, possibly of a serine esterase type, though not

readily inactivated by diisopropyl fluorophosphate and, surprisingly, with the catalytic site in the larger chain⁶³. Proteolytic activity is only apparent when C2 forms a complex with C4 and then its specificity seems to be restricted to hydrolysis of C3; hence the term C3 convertase¹⁴. C2 is also polymorphic and is coded by a gene mapping into major histocompatibility complex in humans⁶⁴.

C3 (refs 65–67) and C5 (ref. 68) are proteins of about 180,000 molecular weight, each containing two chains of about 105,000 and 75,000 molecular weight. Such structural evidence as is available⁶⁹ suggests that C3 and C5 are so similar that, as with C1r and C1s, they probably arose by gene duplication. On activation, both lose a peptide (C3a and C5a) of about 10,000 molecular weight from the longer peptide chain. In each case the small peptide has anaphylactic activity^{70,71}. Only C5a has chemotactic activity⁷². C3, however, is present in much higher concentrations in serum, 1–1.5 g l⁻¹, and is equivalent in concentration to the sum of all the other complement proteins. It seems that C3, as C4, may be synthesised as a single chain precursor molecule⁷³.

Assembly of the component by addition of antibody-antigen aggregates to serum

The mode of assembly of these serum proteins on aggregated antibody and the mechanism of their subsequent activation are far from clear, but several features are beginning to be understood. The antigen seems to have no specific role and whole complement is activated equally well either when antibody is precipitated by addition of a soluble antigen such as ovalbumin, or when antibody-coated red cells are added to serum⁷⁴. Quantitatively, complement consumption per 1,000 molecules of antibody added is 1.5–2 times higher for antibody-coated cells than for antibody aggregates. However, this difference is small and may simply be because antibody molecules spread on a cell surface are more accessible than those in aggregates.

The binding of the first component to the antibody is very efficient (>95% in antibody excess) and activation is complete⁷⁴. C4 is rapidly activated by C1 but only a small fraction (<10%)^{74–76} is bound to aggregates or antibody-coated cells, the rest becoming inactivated in the serum (Fig. 4). Binding of C4 to antibody-coated cells is 10–15 times more efficient than to antibody aggregates, but the number of C4 molecules which become effective as judged by their capacity to form C3 convertase when C2 is in excess is only about twice as great⁷⁴, which is comparable to the difference in C1 binding. This suggests that most of the C4 binds to the cell surface rather than to the antibodies, and little of this is able to form C42 and continue the complement sequence. However, although it has been shown¹⁴ that C4 can bind to red cells in the absence of antibody, it can also apparently cause lysis on the addition of C2 and C1s followed by the later components of complement. The C4 on the cell surface can therefore take part in the activation sequence, presumably by binding C2 and forming C3 convertase. In this experiment, C2 would be formed in solution close to the cell-bound C4. When the antibody is on the cell surface the C1 complex containing C1s would be bound to the Fc part of the antibody and hence the C2 activated at a distance from the cell-bound C4. C2 is rapidly inactivated in serum and in these conditions is likely to be able to interact only with C4 molecules bound on the antibody molecule carrying the C1, or with immediately neighbouring C4 molecules. This could also explain why C4 bound to antibody aggregates is so much more effective than C4 bound to antibody-coated cells.

The activation of C2 is also very inefficient, but quantitative data on the number of activated molecules able to bind and form C42 complexes have not been obtained. A speculative scheme of the assembly of the early components is shown in Fig. 4 and illustrates the binding of C1q to the C_H2 domain of the antibody. The C1r₂-Ca²⁺-C1s₂ complex binds to the collagen tails of C1q and becomes activated⁷⁷. C4 and C2 in solution are activated by C1 and some of the C4 molecules bind either to antibody

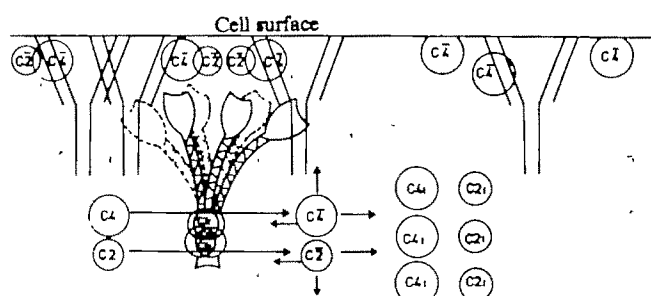


Fig. 4 Suggested assembly of the early components of the classical pathway of complement on antibody bound to a cell surface. The heads of the C1q molecule bind to the C_H2 domain of the antibody and the C1r₂-Ca²⁺-C1s₂ complex to the collagenous tails of C1q through C1r. The activated C1s formed splits C4 and C2. Most C4 and C2 is inactivated in the serum but some C4 binds either to the Fab part of the antibody or to the cell surface. C2 binds to adjacent C4 molecules and forms C3 convertase, but is inactivated before reaching more distant C4 molecules which are ineffective in the haemolytic sequence.

molecules or to the cell surface. C2 molecules which are inactivated rapidly form a C42 complex only with C4 close to the bound C1 complex. The evidence that C4 binds to the Fab portion of the antibody is qualitative⁷⁸ or indirect⁷⁴ and hence is only tentative.

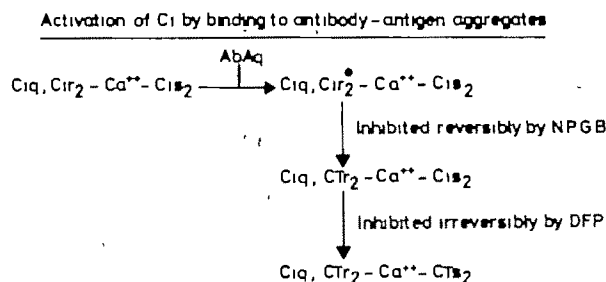
The C3 is split by the C42, and some associates to give C423 which changes its specificity from C3 activation to C5 activation⁷⁹; this is the last proteolytic step in the activation of complement. The activated C5 interacts with C6, C7, C8 and C9 to give the complex responsible for cell lysis and there is evidence that this can bind and lyse cells other than those coated with antibody and responsible for the early activation steps^{80,81}.

Mechanism of activation of the first component of complement

Of the biochemistry of the activation sequence certain mechanisms are clear. For example, in C1 bound or in solution, it is C1s which splits C4 and C2. C1q has no known catalytic activity and no covalent bonds are broken or formed when it interacts with aggregated antibody. C1r will not hydrolyse C4 or C2 in solution nor when bound to antibody aggregates as part of the C1 complex.

Similarly, C1s is not autoactivated when bound or in solution, but it is activated by C1r. It is not clear how C1r is activated²³. Although it is difficult to prepare C1r which is entirely stable at 37°C and neutral pH, repeated addition of proteolytic inhibitors such as diisopropyl fluorophosphate (iPr₂P-F) makes possible the isolation of preparations which are stable over several hours in these conditions. Activation of C1r when bound as C1 onto

Fig. 5 When the tetrameric complex C1r₂-Ca²⁺-C1s₂ interacts with C1q bound to aggregated antibody, the conformation of C1r is changed, exposing a proteolytic site (C1r^{*}). It is this site in the unsplit peptide chain which catalyzes the hydrolysis of a peptide bond and the conversion of C1r^{*} to C1r. C1r activates C1s to C1s^{*}. The active site of C1r^{*} is distinguished from that of C1r in that it reacts reversibly not irreversibly with nitrophenyl guanidine benzoate (NPGb) and it is not inhibited by diisopropyl phosphite fluoridate (DFP).



antibody aggregates occurs in 5–10 min, so satisfactory controls can be obtained³¹. It is then found that although C1q and C1r will bind firmly to antibody aggregates in the presence of Ca²⁺, no activation occurs. C1s in the complex is essential for C1r activation but C1r or C1s inactivated with iPr₂P-F (iPr₂P-C1s) can replace C1s. As iPr₂P-C1s is effective in facilitating the activation of C1r, C1s can have no proteolytic role in the activation. Presumably, therefore the binding of the C1r₂-Ca²⁺-C1s₂ complex is necessary because it determines the conformation of C1r. In the presence of 10 mM iPr₂P-F, C1r is still activated but C1s is not. As both C1r and C1s are inactivated readily by iPr₂P-F this agrees with the evidence that C1r activates C1s but suggests that C1r will not activate C1r in the complex, as is found in solution³¹.

Activation of C1r and C1s in the complex is inhibited if 1 mM nitrophenyl-guanidino-benzoate is present, but the inhibition is reversed on removal of this compound. Nitrophenyl-guanidino-benzoate will inhibit C1r and C1s irreversibly and hence again it is unlikely that C1r activates C1r (ref. 31). However, the kinetics of C1r activation show a sigmoidal curve suggesting autocatalysis. The most likely explanation of these results (Fig. 5) is that the binding of C1r₂-Ca²⁺-C1s to the C1q on the antibody causes the C1r to take up a conformation which exposes a catalytic site in the unsplit C1r molecule³¹. This catalyses hydrolysis of C1r to C1r which in turn activates C1s.

Activation of components C4, C2, C3 and C5

C1s then hydrolyses C4 with loss of a 6,000 molecular weight peptide chain from the α chain and also splits the C2 peptide chain into 70,000 (C2a) and 30,000 (C2b) molecular weight chains³². The activated C4 binds either to the Fab portion of the antibody or to the cell surface and interacts with such activated C2 molecules as reach it before they are inactivated. Inactivation of C2 seems to be continuous in terms both of loss of ability to form C42 and in loss of C3 convertase activity after formation. When C2 interacts with C4 covalently attached to Sepharose and is then treated with C1s, the 70,000 molecular weight fraction, C2a, dissociates leaving C2b still bound. It has been suggested that the interaction with C4 is through the C2b part of the molecule and loss (50% in 10 min) of C3 convertase activity may be due to the dissociation of the C2a which probably contains the catalytic site, from the complex⁶⁰. Characterisation of the catalytic site and explanation as to how interaction with C4 determines its specificity will be necessary for an understanding of the mechanism of these reactions.

The subsequent step of the formation of C5 convertase by interaction of activated C3 with C42 to give C423 is even less well understood, but investigation was made possible by the finding that treatment of human C2 with weak iodine solution produced a 10–20-fold increase in its stability and activity in forming C3 convertase⁶³. Centrifugation in a sucrose density gradient of C42 using iodine-treated C2 and C3 showed the presence of two peaks of C5 convertase activity of 14–16 S and 9–10 S and a peak of C3 convertase activity at 11.3 S (ref. 84). It was suggested that the heavy C5 convertase activity could be explained by the presence of an equimolar C423 complex and the lighter by an overlap of the C42 and C3 peaks. If this is correct, noncovalent binding of C42 and C3 is not essential for C5 convertase activity, but much more detailed investigation will be necessary to establish the nature of this enzymatically active complex.

This is the last proteolytic step in complement activation and no catalytic activity has been found in activated C5 or in the lytic complex it forms with C6, C7, C8 and C9. Of the four proteases of the classical system, C1r, C1s, C42 and C423, the first two are clearly of the serine esterase type and the two enzyme complexes, where the catalytic site is believed to be in C2, may also be of this type. However, it is unusual in that it is relatively resistant to diisopropyl fluorophosphate and, more surprisingly, the active site seems to be in the 70,000 molecular weight chain⁶³, whereas in all other serine esterase type proteases it is in chains of 20,000–30,000 molecular weight. All these proteases

of the classical pathway are active in solution but are probably bound to antibody or cell surfaces *in vivo*. This is likely to be an essential feature, as plasma and other body fluids contain high concentrations of proteolytic inhibitors and activation must depend on the local excess of protease over inhibitors.

There are many finely balanced features of this highly complex system, the ramifications of which are far from clear, and the unravelling of the mechanisms of activation and control seem likely to throw new light on an animal's immune defence against infection.

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articles

High energy neutrino astronomy

David Eichler & David N. Schramm

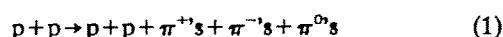
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The possibility of doing point source neutrino astronomy is discussed. Probable sources include galactic nuclei, Seyferts, quasars, radio galaxies, pulsars and supernovae.

THE possibility of building a very large deep underwater muon and neutrino detector (DUMAND)¹, which would look at the interactions of neutrinos produced by cosmic rays hitting the atmosphere has been discussed. New design ideas mean that this detector may be able to resolve the direction of origin of the neutrinos to better than 1 deg² which opens up enormous possibilities for point source neutrino astronomy some of which are considered here. These may not need to wait for DUMAND but may be realisable with existing detectors of the type being developed by Lande². For most of our discussion, however, we will use proposed parameters for DUMAND; such that a flux from a point source of order 10² eV cm⁻² s in neutrinos more energetic than 3 × 10¹¹ eV constitutes a detectable signal for a 10⁹ ton detector in one year of viewing time. (This flux produces 10 counts per yr per 10⁹ tons.) Signals as small as 10 eV s⁻¹ cm⁻² may prove to be detectable due to solid angle resolution and proper identification of background and effective detector masses larger than 10⁹ tons. The 10⁻² eV flux level is not large by astrophysical standards: the brightest non-thermal sources (such as pulsars and active galactic nuclei) have fluxes far greater than this, so if they can somehow put even a small fraction of their total luminosity in the form of high energy neutrinos, they are in principle detectable.

Any system which has high energy proton collisions will produce neutrinos due to pion and kaon production and decay in such collisions.

The production of high energy neutrinos in astrophysical systems thus entails first the production of high energy protons, followed by their degradation through pion and kaon producing collisions against other protons^{3,4}. The most important astrophysical reaction is



While the neutral mesons decay into γ rays, the charged pions eventually decay into three neutrinos and an electron (or positron) by the standard decay sequences. The cross section for proton collisions is roughly 40 mb, and is only weakly dependent on the collision. Thus protons are stopped over a path length of about 30 g cm⁻². The leading pion may contain a significant fraction of the collision energy, thus a few neutrinos may carry away a large fraction of the collision energy⁵.

Pions can also be produced in proton-photon collisions when the photon has an energy >600 MeV in the frame of the proton.

The cross section for this reaction depends on the collision energy and at maximum is

$$\sigma_{\pi\pi} \approx 2 \times 10^{-28} \text{ cm}^2 \quad (2)$$

Diffuse sources

High energy galactic cosmic rays hitting the upper atmosphere will produce high energy neutrinos. These are the prime neutrinos which the high energy physicist might use for directly probing the nature of the weak interaction, and searching for intermediate vector bosons and Higgs particles. This diffuse atmospheric flux will be higher for those neutrinos coming from the horizon than from overhead due to the relative (atmospheric) decay length. In addition, at high energies this flux will drop off with energy by an additional 1/E due to time dilation on the decay of the π 's and k 's preventing neutrino production. The average intensity of this background in the energy range of interest, 10¹²–10¹⁴ eV, is <0.3 counts deg⁻² yr⁻¹. These considerations indicate that this atmospheric background should be easily distinguished from neutrino fluxes of extraterrestrial origin. (Direct neutrino production⁶ will not have the additional 1/E drop off but it is down $\sim 10^{-4}$ relative to π and k production.)

Berezinsky and Zatsepin⁴ and Margolis *et al.*⁴ have noted that the galactic cosmic rays interacting with the interstellar matter will produce a background which is marginally detectable and is peaked towards the galactic centre. Here, as in all astrophysical sources, all of the π 's and k 's decay so the ν -spectral index is essentially the same as that of the high energy protons with no additional 1/E time dilation effect.

Other galaxies will similarly contribute to the diffuse ν -background^{3,4}; however, if the other galaxies have the same galactic cosmic ray intensity and spectral shape as ours, then ours will dominate the diffuse ν -background. On the other hand, it is reasonable to expect that galaxies were more energetic in the past and thus produced more ν 's. Berezinsky and Zatsepin⁴ mentioned the possibility of a very early 'bright phase' of galaxies to produce very large numbers of ν 's. These ν 's will, of course, be redshifted, but they may, nevertheless, dominate the diffuse background. Thus, a measurement of the diffuse background alone would enable us to make some discrimination between a bright early phase for galaxies and the situation where early galaxies are similar to our present one.

Point sources

For the astronomer, the most appealing aspect of a large neutrino detector, such as DUMAND, is the ability to resolve

individual point sources. As mentioned earlier, a variety of non-thermal systems would be detectable if they generate a significant fraction of their luminosity in high energy neutrinos. To do so, a system must, (1) produce fast particles efficiently; and, (2) contain enough target matter near the source of the fast protons to stop a significant fraction of them. Many of the most exciting objects in the sky may meet these criteria. Table 1 lists several astrophysical situations in which both these conditions may be met.

Supernovae

Standard cosmic ray models assume that a typical supernova puts out 6×10^{50} erg in relativistic cosmic rays. With the spectrum proportional to $E^{-2.5}$. The neutrino counting rate in a 10^9 ton detector is then⁶

$$R = 10^{-3} n / D^2 \text{ d}^{-1} \quad (3)$$

where D is the distance in kpc to the supernova remnant and n is the ambient density in atoms cm^{-3} of the medium in which the remnant is situated. Regardless of the detailed mechanism by which the cosmic rays are accelerated in either the remnant or the shock itself, the basic fact remains that if the supernova occurs in a dense cloud where η may be as large as 10^3 , it would then be a detectable point source of high energy neutrinos. Because supernovae are presumably rapidly evolving massive stars, it is reasonable to assume that many go off while still in the immediate vicinity of the cloud out of which the presupernova star formed. It is also likely that a presupernova star will undergo significant mass loss and hence create its own circumstellar cloud.

A supernova in a dense environment would also be detectable by γ -ray satellites in the energy range $E_\gamma < 1$ GeV. Indeed, coincidences have been reported⁷ between unidentified γ -ray sources detected by the Cos B satellite and radio emitting supernova remnants. The signal detected by Cos B, if assumed to be the low energy end of an $E_\gamma^{-2.5}$ γ -ray spectrum originating from π_0 decay, would yield 10 – 10^2 $\text{eV cm}^{-2} \text{ s}^{-1}$ in 10^{12} eV γ -rays, and a comparable flux in high energy neutrinos.

Pulsars

Pulsars accelerate high energy protons⁹ which if they hit something will produce neutrinos. Berezhinsky⁸ has pointed out that young pulsars, blanketed by a supernova remnant less than a year old could be bright sources of high energy neutrinos. However, their behaviour at such early times is highly uncertain. Despite these uncertainties, it is reasonable to believe that a pulsar in a binary system could be a source of high energy neutrinos for the first few thousand years of its existence¹⁰. The stellar wind of the companion could have a column density as high as 1 – 10 g cm^{-2} , its corona could provide further target material and an accretion cloud could conceivably surround the pulsar. If the companion is a red giant, its envelope could block

nearly half the high energy protons emitted by the pulsar. A pulsar emitting (roughly the luminosity of the Crab pulsar) $10^{37} \text{ erg s}^{-1}$ in high energy particles ($E_p > 10^{13}$ eV) at a distance of D kpc produces

$$R = \eta D^{-2} 10^2 \text{ counts d}^{-1} \quad (4)$$

where η is the fraction of the luminosity converted to high energy neutrinos by the target material near the pulsar. A typical value of η might be between 10^{-3} and ~ 1 .

Helfand and Tademaru¹¹ have speculated, on observational grounds, that a significant fraction of all pulsars form in binaries. Stepnian *et al.*¹² have detected a flux in high energy γ rays ($E_\gamma > 3 \times 10^{12}$ eV) of about $60 \text{ eV cm}^{-2} \text{ s}^{-1}$ from Cyg X3, which is generally held to be a (heavily blanketed) pulsar in a binary system. A comparable flux in high energy neutrinos would be detectable with a 10^9 ton detector in a year or two of viewing time. Any process—such as Colgate's¹³ shock mechanism—which produces high energy particles promptly during the initial supernova explosion will produce a prompt burst of neutrinos if the exploding star has a binary companion. For example, in the type I supernova model where a white dwarf accretes beyond the Chandrasekhar limit from a red giant companion, the explosion will, of course, occur in the vicinity of that companion. The narrow pulse detected at Earth would contain a maximum of

$$N = \eta 10^8 D^{-2} (U/10^{48} \text{ erg}) \text{ counts}$$

where U is the energy output of relativistic particles, and η is the fraction of them stopped by the binary companion and surrounding material. A typical value here for η might be as high as 0.5.

Galactic nuclei

The most potent sources of high energy neutrinos may be active galactic nuclei¹⁴, such as those of radio galaxies, Seyferts and possibly quasars. Such nuclei are capable of luminosities as high as 10^{43} – $10^{47} \text{ erg s}^{-1}$. As the radiation seems to be of non-thermal origin, it is believed that the power outputs in high energy particles take on similar values. As these high energy particles would be produced within the innermost regions of the nucleus, which have column densities well in excess of a typical galactic disk, the high energy protons produced there might be expected to be stopped by nuclear collisions before escaping the nucleus. (Consider, for example, that cosmic rays in our own galactic disk go through a few g cm^{-2} of matter before escaping. Were the column density of the disk a factor of 30 higher, the dominant loss mechanism would be nuclear collisions.) Moreover, it can be argued¹⁵ that the accretion column surrounding a collapsed object in the nucleus may have column densities of 10^2 – 10^3 g cm^{-2} , which would be sufficient to even stop the γ rays, but the ν 's would still easily escape.

Thus, one could reasonably expect that such active nuclei put out a significant fraction ($\geq 10\%$) of their entire luminosity in the form of high energy neutrinos. The nearby peculiar galaxy M82 (often thought to be an exploding galaxy because of its optical appearance) could give a neutrino count rate in excess of 1 per day if, indeed, there are nonthermal processes occurring in its nucleus. Other nearby active nuclei, such as Cen A and M87 could give count rates of the order 10 per year. Grindlay *et al.*¹⁵ reported a high energy ($E_\gamma > 3 \times 10^{11}$ eV) γ -ray flux of the order of $50 \text{ eV cm}^{-2} \text{ s}^{-1}$ from Cen A. A comparable flux in high energy neutrinos would be detectable within a year of viewing time. (Remember that the atmospheric background per deg in a 10^9 ton detector is $\leq 0.5 \text{ counts yr}^{-1}$ thus as few as 3 counts yr^{-1} from the same deg would indicate a point source.)

Compact radio sources emit in bursts which almost certainly originate within regions smaller than a light day. Thus, a few years worth of neutrinos as computed from average luminosities could arrive within a few hours, making them much more easy to detect. It has been suggested¹⁶ that as many as 10^4 counts per 10^9 tons per burst could be recorded from a radio burst, for example, the nucleus of 3C120.

Table 1 Possible high energy neutrino sources

Site of He Particle production	Target material	Maximum expected counting rate from a typical source
Expanding supernova remnants	Ejecta, swept up matter, dense cloud from which star formed	10^2 per yr per 10^9 tons
Stellar explosions	Binary companion	$10^8/10^9$ tons per event
Pulsars	Binary companion, accreting matter young supernova remnant	10^3 per yr per 10^9 tons
Seyferts, radio galaxies, and QSOs	Clouds in nucleus, matter accreting on to compact object	10^3 per yr per 10^9 tons

Particularly significant is the fact that $\sim 10\%$ of the flux from Cen A is in the form of high energy γ rays. This corroborates the picture sketched above, that is that a significant fraction of the luminosity of a non-thermal galactic nucleus is caused by very energetic particles. The energy density in the Universe due to emission by Seyferts and QSOs has been conservatively estimated at $10^{-3} \text{ eV cm}^{-3}$. If Seyferts do emit $\sim 10\%$ of their luminosity in the form of high energy neutrinos, the energy density in such neutrinos could be as high as $10^{-4} \text{ eV cm}^{-3}$. Thus, one could reasonably hope to discover a cosmic background of high energy neutrinos standing out above the atmosphere at $E_\nu > 10^{12} \text{ eV}$.

Limits on deuterium production

Another area where neutrino astronomy may be critical is in constraining the astrophysical locations for producing deuterium¹⁷. If deuterium is only produced cosmologically then the Universe is open (see ref. 18 and refs therein). Epstein *et al.*¹⁹ showed that the only allowable alternative to the big bang for producing deuterium was extremely high energy particle interactions where most nuclei (in particular Li, Be and B) would be broken down to free nucleons. To avoid seeing the simultaneously produced γ 's from such interactions, any such production site must be hidden in an optically thick region (for example, Epstein²⁰ proposed the centres at quasars), however, neutrinos cannot be hidden in this way. Thus, limits on the neutrino flux would constrain, if not eliminate, any non big bang deuterium production site. The Reines experiment²¹ implies that the cosmic background at $E_\nu < 10^{12} \text{ eV}$ is well below the atmospheric background, and it can be shown²² that this constrains Epstein's model for deuterium production to $1 + Z > 2000(A^2\Omega)^{-2/3}$.

Conclusions

Many of the most exciting objects in the sky may be high energy neutrino emitters. Any object that emits γ 's by π^0 production will also naturally be a neutrino source. Thus, γ -ray astronomy gives us some degree of confidence that neutrino astronomy will not be a null field. However, the two fields are complimentary and will not merely duplicate each other because neutrinos are capable of being seen even when they are produced in regions which are optically thick to γ rays. For example, the very compact central regions of active galaxies, with their intense radiation fields, will be optically thick to γ -rays but can be

studied by neutrino astronomy. In probing these very central regions, one may discover how these objects are powered. In fact, even a null result is interesting because it would indicate the degree to which high energy particles are involved. In addition, γ -ray detectors mostly tend to be sensitive to lower energies, thus seeing an object in both ν 's and γ 's will tell us a lot about its high energy particle spectrum. (The mean free path of γ 's of energy $\geq 10^{14} \text{ erg}$ is $\approx 10^4 \text{ kpc}$).

One final advantage of ν -detectors is that they simultaneously observe a full 4π steradians. Thus they will not miss transient point sources. This feature may enable ν -detectors to alert other branches of astronomy to transient objects.

Whenever astronomers have opened a new channel for studying the Universe, new and unexpected sources have been discovered. One only has to consider, quasars and pulsars from radio astronomy, Cyg X1 from X-rays, and the still mysterious X-ray and γ -ray bursters to realise that the most exciting possible discoveries from ν -astronomy may not have been mentioned here.

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Rifting, crustal attenuation and subsidence in the Bay of Biscay

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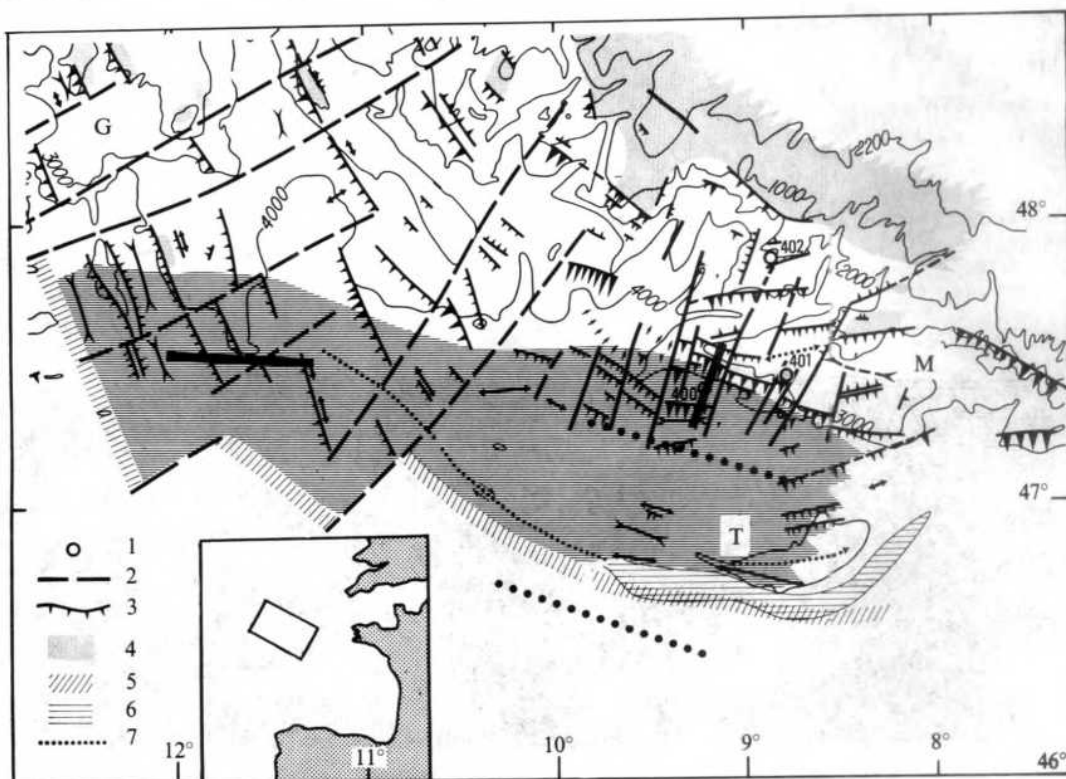
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The distribution of the Tertiary and Lower-Upper Cretaceous sediments in part of the northern continental margin of the Bay of Biscay, has been mapped. Three sedimentary sequences are indicated by the seismic profiles.

THE part of the northern continental margin of the Bay of Biscay discussed here intersects and lies seawards of the rifted Mesozoic-Cainozoic basin and Cornubian granite platform of the western approaches to the English Channel. The continental

slope is broad and marked by prominent features such as the Meriadzek Terrace, Trevelyan Escarpment and the Goban Spur. The structure of the margin consists of thin and unfaulted, Tertiary and Lower-Upper Cretaceous sediments that mantle a series of tilted and rotated fault blocks¹ underlain by a thinned continental crust^{2,3}. The distribution of these sediments and the fault blocks has been mapped using 10,000 km of multichannel seismic profiles occupied by the Institut Français du Pétrole (IFP-CNEXO-CEPM) and the Institute of Oceanographic Sciences for the Department of Energy (UK). These data were

Fig. 1 Schematic tectonic pattern of the rift system in NE Biscay: 1, sites 400, 401, 402; 2, faults; 3, lystric faults with arrows down dip; 4, Pre-Aptian erosional surfaces; 5, continental oceanic crusts boundary; 6, tectonised area along Trevelyan escarpment due to late Eocene compression; 7, late Eocene strike-slip fault or folds. Heavy dotted line, seismic refractions profile; heavy solid line, seismic reflection profile Figs 2a; 3. Horizontal hachures, areas where deep reflector 'S' can be seen.



acquired using 24-channel common depth point reflection techniques and processed using deconvolution before and after stack followed by the application of time and space variant filtering. Diffraction migration has also been applied to clarify reflections.

The seismic profiles show that the tilted and rotated blocks are bounded by lystric faults which delineate half-grabens created during rifting and subsequently covered by post-rift sediments^{1,4}. Three sedimentary sequences can be recognised on the seismic profiles. A pre-rift series which consists of thick layered sediments shown by parallel inclined reflectors is clearly visible within the fault blocks. Evidence for the age and depositional environment is given by drill and dredge data⁴⁻⁷. Site 401 of DSDP Leg 48 (refs 4, 5) penetrated Kimmeridgian-Portlandian limestones in the crest of one fault block although the regularity of the pre-rift reflectors (Fig. 2a) hints that substantial facies changes may be present. Dredging on exposed fault blocks at 4,200 m depth near Goban Spur, however, has yielded granites of Hercynian age⁷ and pebbles of Permo-Triassic (?) sandstones have been noted in Lower Cretaceous conglomerates⁶. The second syn-rift sequence, deposited contemporaneously in the half-grabens, is inferred to be pre-Aptian and probably post-late Jurassic⁴. The post-rift sequence penetrated at DSDP sites 400A, 401 and 402A was deposited after the end of rifting and the onset of spreading in Aptian time. It comprises thin pelagic or hemipelagic sediments of post-Aptian age that drape the underlying fault block topography⁴.

The tilted and rotated fault blocks can be followed from the shelf break to the outer part of the rise in the vicinity of the Trevelyan Escarpment at 4,500 m depth. The polarity of the faulting is consistently down towards the continent-ocean boundary and true horsts are rare. The fault blocks (Figs 1, 2a) typically trend WNW-ESE subparallel to the margin but their continuity is limited due to normal faults whose NE-SW and E-W trends may reflect the influence of older Hercynian structures such as the Armorican shear zones. Towards the Goban Spur, the fault blocks change trend to NNW-SSE and are paralleled by the magnetic anomalies of the adjacent ocean crust⁸. In both areas, the position of the continent-ocean boundary (Fig. 1) is clearly shown by a sharp change to a strongly diffracting opaque reflector that contrasts with the tilted reflectors observed within the fault blocks.

The fault blocks are typically spaced at between 10 and 30 km (Figs 1, 2a) and the bounding fault planes curve and flatten with depth. Throws of individual faults may be as much as 3-4 km. Comparable lystric faults have been described from the Viking Graben of the North Sea^{8,9} and from the Gulf of Suez rift¹⁰. Beneath the irregular surface defined by the crests of the fault blocks, a prominent reflector 'S' (Fig. 2b) is observed at depths between 9.0 and 11.0 s beneath the rise. Variations in the travel time to reflector 'S' beneath and between the fault blocks are produced by lateral variations in velocity. It may therefore be assumed that reflector 'S' is sub-horizontal and thus independent of the overlying faulted layer. Indeed, we have not observed any displacement of reflector 'S' even though the curved normal faults are associated with throws of as much as 3-4 km. An exactly similar feature observed at the western foot of Galicia Bank to the west of Portugal shows that reflector 'S' is not a local phenomenon (Fig. 4).

Further evidence on the nature of reflector 'S' is given by a refraction profile which crosses our seismic reflection profiles and was obtained by CNEXO using OBS (ref. 2). The base of the 4.9 km s⁻¹ layer is 9.5 km, close to the depth at which the lystric faults become horizontal. Below this layer, a 3-km thick layer with 6.3 km s⁻¹ occurs above the Moho (8.2 km s⁻¹). The refraction data indicate that the interface between the 4.9 and 6.3 km s⁻¹ layers lies at ~9.2 s, in reasonable agreement with the observed depth of reflector 'S' (Figs 2b, 3). The structure of the faulted layer is, however, complex, and horizontal inhomogeneities may be present. In view of this and the small 3-km thickness of the 6.3 km s⁻¹ layer the possibility exists that reflector 'S' may be the Moho. Clearly, further seismic studies are necessary.

Rifting and crustal attenuation

Experimental simulation of rifting using clay models has clearly shown that normally faulted tilted blocks are formed in response to crustal tension¹¹. The accommodation of these movements at depth bears directly on proposed mechanisms of crustal attenuation during rifting. One widely used model of rifting is based on analogues such as the East African rift system or Rhine graben.

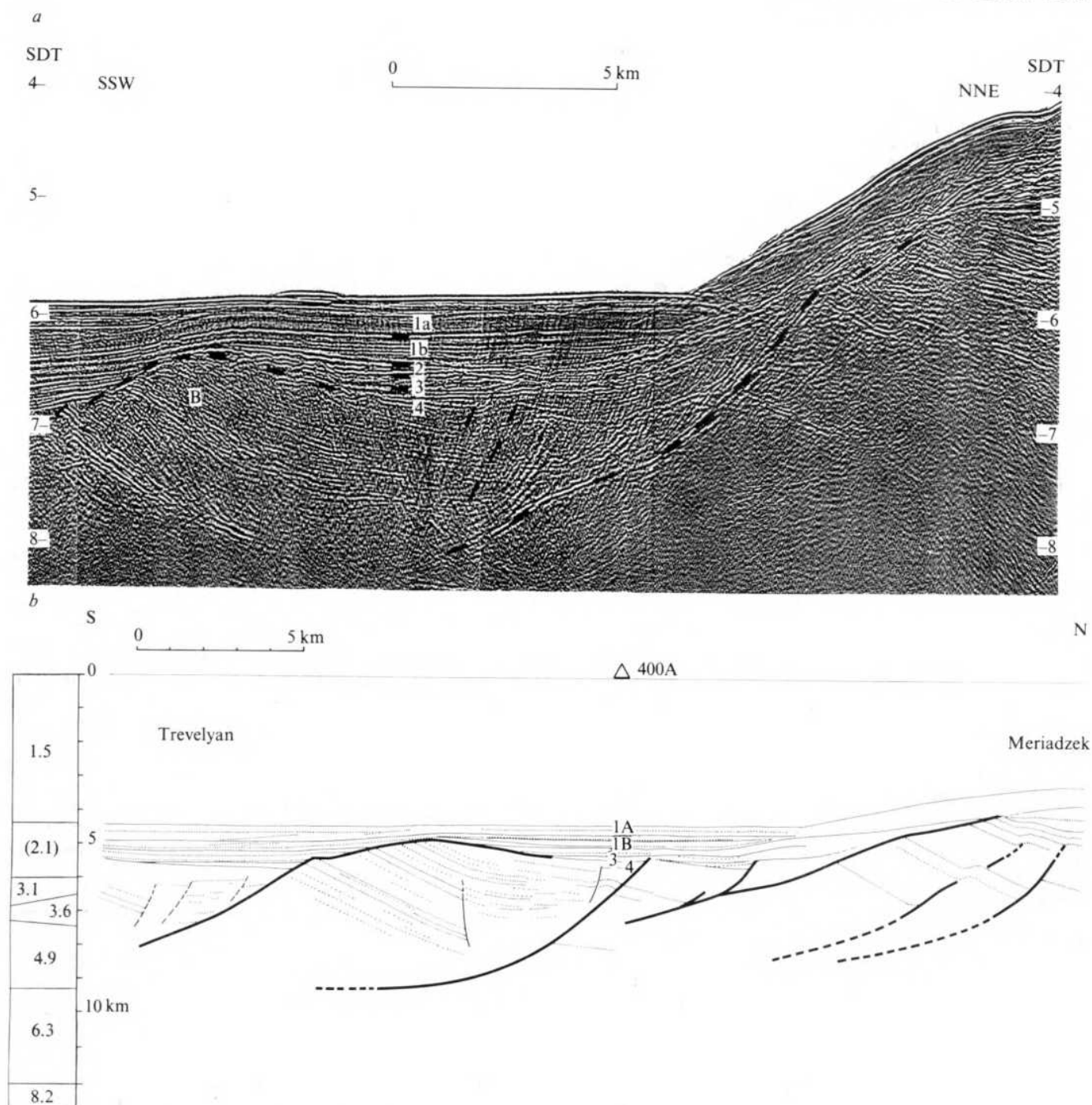


Fig. 2 *a* Tilted block and lystric fault. Acoustical basement B is constituted in a great part of layered sedimentary rocks of probable Jurassic and early Mesozoic age. These formerly continuous layers were faulted and tilted during the early Cretaceous rifting phase. Vertical scale in seconds, two-way travel time. Numbers 1a, 1b, 2, 3, 4 refer to overlying sedimentary formations: Profile OC 412, migrated; Site 400. *b*, Geological cross section through site 400 with same horizontal and vertical scale, constructed from seismic reflection—profile OC 412 migrated (see Fig. 1). Note the lystric faults bounding the tilted blocks. Near base of lystric faults is an horizontal reflector corresponding to interface between 4.9 km s^{-1} and 6.3 km s^{-1} layers defined by seismic refraction data. Moho discontinuity is at 12 km (ref. 24).

The following stages have been proposed¹²: upwarping or doming of the crust, incipient volcanic activity, formation of faults and increasing volcanic activity, subsidence of the graben(s). Other workers^{13,14} proposed that domal uplifts developed in isostatic response to mass deficiencies produced by partial melting of the mantle above rising plumes at the base of the lithosphere. In these models, sub-aerial uplift and erosion with 'sub-crustal erosion' are offered as a means of thinning the crust. The second and fundamentally different mechanism pro-

poses a combination of extension by brittle fracture in the upper part of the crust and ductile flow in the lower part of the crust¹⁵⁻¹⁹. Important constraints can be placed on these by the regional geology of the margin and also by the geometry of the extensional fault fabric.

In the Bay of Biscay, sub-aerial uplift which preceded rifting is not evident, in contrast to the classical models⁴. Rifting, which probably took place between late Jurassic and Intra-Aptian time⁴ occurred in a pre-existing epicontinental sedimentary

basin of Mesozoic age perhaps first formed during earlier Permo-Triassic distension^{5,9}. The seismic profiles indicate that no erosion occurred before and during rifting on the outer part of the margin, that is, in the axis of the rift system. Dredge data^{6,7}, the drilling results from Leg 48 (refs 4, 6) and the seismic data indicate that at the end of rifting, water depths were of the order of 2,000 m in the outer part of the margin. The existence of deep water marine beds implies that rifting was not associated with a substantial sub-aerial relief—a conclusion that can also be drawn for the Parentis Basin in south-east Biscay²⁰. We suggest that the rifting was not preceded by and is thus not a consequence of a domal uplift. Beneath the inner part of the margin, however, the crests of some tilted blocks are cut by a horizontal plane, indicating that some sub-aerial erosion did take place when the fault blocks were rotating.

The importance of normal faulting in thinning the crust has been more contentious, for little has been known of the geometry of such faults at depth and their relationship to the deeper structure of passive margins. The new data (Figs 2b, 5) clearly show that the lystric faults do not continue as far as the base of the crust but curve and flatten with depth demonstrating that extensional faulting is confined to the upper part of the crust. Such faults are considered to form by simultaneous faulting and tilting so that the fault blocks rotate towards the central subsiding trough and individual blocks dip away from the rift

if high pore fluid pressures are absent²², it seems unlikely that these would develop under an extensional stress regime.

Our hypothesis considers that the termination of faults with depth marks a fundamental transition in the rheological properties of the crust from brittle above to ductile below^{19,23,24}. The boundary or transition between them may be marked by the deep reflector 'S' so that the interval between the lystric faults and the Moho corresponds to a zone of ductile flow characteristic of the lower part of the crust. The hypothesis implies that there is a depth in a homogeneous crust subject to extension below which ductile behaviour occurs and above which elastic strain is released by periodic faulting. Flow within the ductile layer during rifting, that is, while the continental crust remains joined, is considered to accommodate the balance of the thinning required by the geological and geophysical data.

Our observations of rift tectonics in the Bay of Biscay support modifications to the Vening Meinesz hypothesis of rift valley formation made by various authors^{15-17,21,23-25}. Intraplate stresses arising from continental collisions or from small differences between the absolute velocities of plates may initiate stretching of the lithosphere^{10,14}. These stresses are imposed on a crust consisting of the brittle upper layer and a lower ductile layer that can deform rapidly when stress differences are large. As shown above, the limited extension and thinning produced by brittle fracture compared to the more important ductile thinning of the

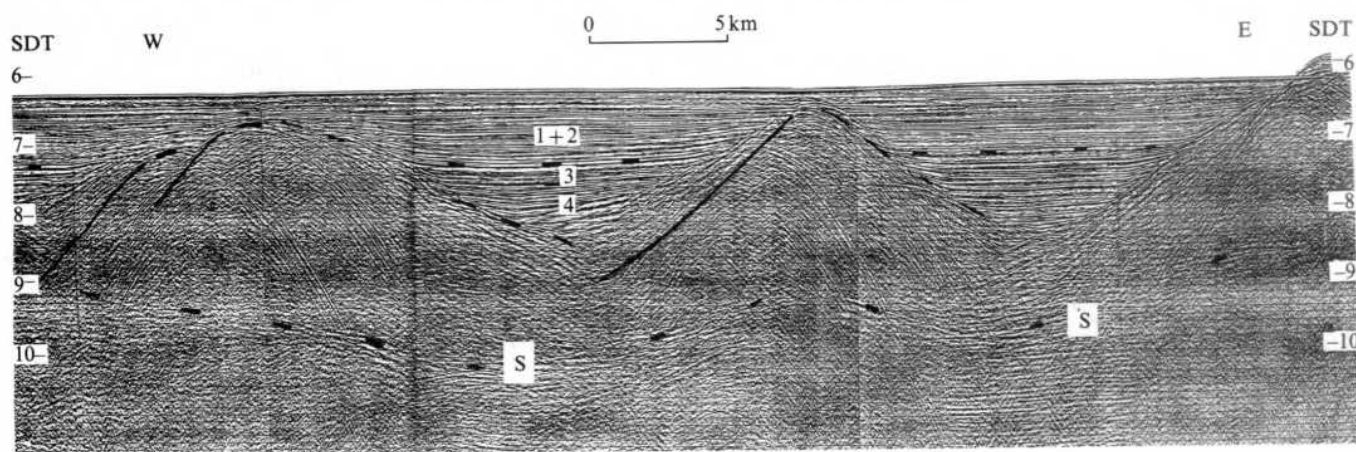


Fig. 3 Seismic profile south of Goban Spur showing tilted blocks with lystric faults. Note the horizontal reflector S below the tilted blocks. It is observed on the deepest part of the margin. On Trevelyan it corresponds to the boundary between a 4.9 km s^{-1} layer and a 6.3 km s^{-1} layer only 3 km thick. The Moho is around 12.5 km below sea level. Profile CM 16, processed.

axis²¹. Beneath the outer part of the margin, the continental crust affected by lystric faults is $\sim 6 \text{ km}$ thick although rotation of the blocks reduces this thickness to about 4–5 km (Figs 2b, 5).

As further fault movement becomes difficult when the fault plane becomes horizontal, we have roughly compared the crustal thinning and extension derived from the variable fault geometry with that given by gravity models and refraction data^{2,3}. For the typical rotation of $20\text{--}30^\circ$, extension of the area between the present shelf edge and the continent-ocean boundary is $\sim 10\%$ of the previous width. The rotation data also indicate a thinning of between 6 and 9 km (after ref. 21). The refraction data show, however, that the crust has thinned to 6 km (that is by 24 km assuming an original thickness of 30 km) beneath the outer part of the margin^{4,5}. We therefore conclude that extensional faulting in the upper part of the crust during rifting is inadequate to account for the observed thinning.

Clearly, the lower part of the crust must also be thinned but by some other mechanism. We have considered an origin by decollement, although this is not preferred, as both gravity and high frictional drag must be overcome to allow movement along a subhorizontal zone dipping toward the continent. Furthermore, the direction of movement would be opposite to that taking place during extension. Although frictional drag can be reduced

lower crust suggests decoupling between the brittle and ductile parts of the crust. Experiments in rock mechanics³⁰ have shown that the strain of ductile material increases continuously once a certain level of stress is reached (viscous behaviour). In contrast, continuous strain in the brittle material is limited because stress increases only until released by fracturing. These differences in rheological behaviour may imply that thinning of the ductile part of the crust began before the creation of the first fault in the brittle crust. As tensile stresses are the same at the lateral boundaries of the lithosphere, it follows that extension by continuous viscous flow initiated by a lower level of stress in the lower ductile part is more important than in the upper, brittle part. Normal faults develop as the strength of the brittle layer is exceeded and downfaulting results in a decrease in tensile stress and elastic shortening that equals the extension caused by faulting. Continued viscous flow in the ductile layer results in the accumulation of stress in the brittle layer that is relaxed by further faulting. The continuous necking of the lower ductile part of the crust is accompanied by a rise of the mantle and a development of a depression, the rift, in the brittle upper crust that allows continued rotation of the fault blocks along the lystric faults. It is important to realise that the mechanism allows greater thinning within the ductile layer than in the brittle layer

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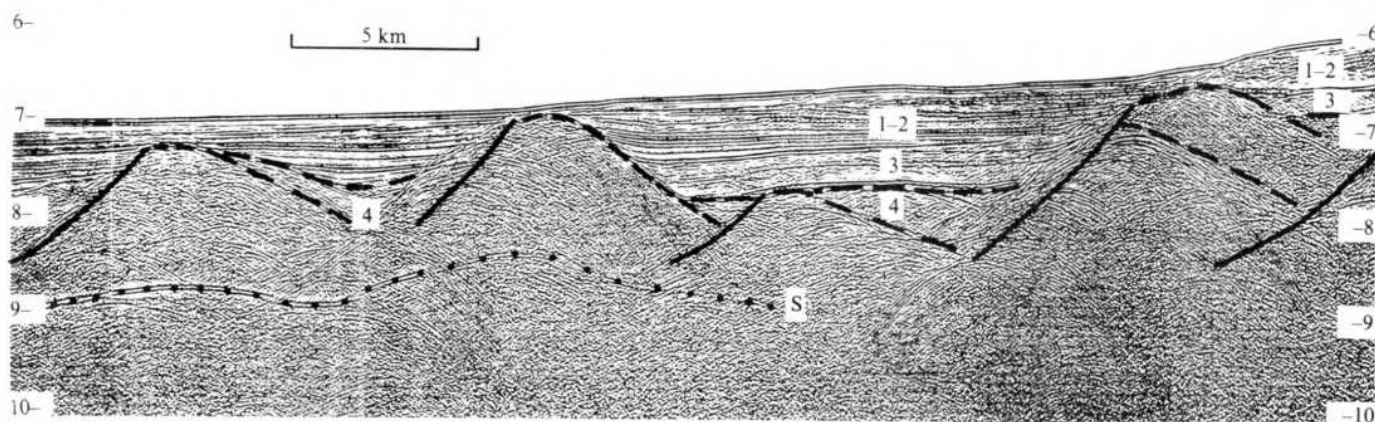


Fig. 4 Seismic reflection profile immediately west of Galicia Bank showing tilted blocks and lytric faults and an horizontal reflector below, as on Fig. 1(7); seismic formations 1-4 are the same as defined in Northern Biscay. Profile IFP-CNEXO-CEPM GP12 processed.

because of the difference in rheological properties. Extension within the brittle layer should not be regarded as a measure of thinning which largely takes place within the ductile layer.

Models suggested for the Baikal rift and Rhine Graben^{15,16} fit our observations well. Heat flow data for the Biscay margin²⁷ can only be interpreted in terms of thinning of the ductile lower part of the continental crust and not in terms of phase changes or intrusions. Rim uplifts predicted by the model may be due to regional isostatic adjustment indicated by the erosion of the fault blocks observed near sites 401 and 402 and more widely on the shelf. In Biscay, Galicia and the Aquitaine Basin^{1,4,20,26}, the regional geological data indicate that the rifting took place in a pre-existing epicontinental basin subject to subsidence and perhaps weakened during earlier rifting episodes. In these cases, previous crustal thinning which may have occurred in response to sediment loading as well as rifting may have subdued the development of complementary uplifts or erosion may have kept pace with uplift. The faulting may have been rapid although we cannot be specific on this point. For example, onlap of reflectors within the bedded sequence contained within the fault blocks is rare but thickening towards the axes of the half graben is common. This observation is indicative of relatively rapid and continuous contemporaneous movement rather than episodic movement.

Post-rifting subsidence

Regional subsidence of passive margins beginning at the onset of spreading and after rifting is a widely recognised phenomenon. In Biscay, post-rift subsidence of 2,300 m indicated by shallow water Aptian-Albian sediments was comparable to the subsidence produced by rifting^{4,5}. This subsidence was not caused by faulting, for the post-rift sediments are only rarely affected by faults. These exceptions are faults of late Eocene age developed along the Trevelyan Escarpment in response to the Pyrenean compression. The post-rift subsidence therefore took place by oceanwards tilting of the margin without decoupling of the continental and oceanic crust. Subsidence due to loading can be

neglected in Biscay as the post-rift sediments are thin (Figs 2b, 5).

The most widely accepted mechanism of post-rift subsidence is thermal contraction of the lithosphere initiated as the heat source migrates away from the margin at the onset of spreading; the exponential decrease in subsidence with time is considered to be similar to that deduced for the ocean crust^{4,28}. The depth of deposition of Aptian sediments (Fig. 6) that is, of the topography of the margin at the end of rifting, has been reconstructed from the new seismic and palaeobathymetric data⁴. Although the present altitude of a point on the margin depends on its initial altitude, the curve shows that the subsidence increases progressively from the shelf towards the deep ocean. Variations in

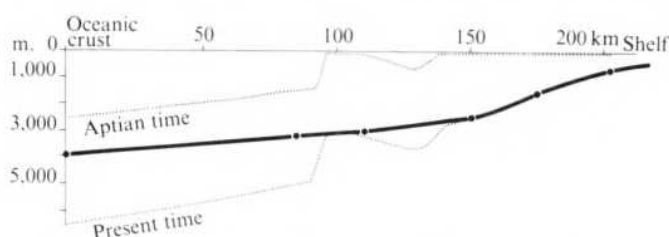
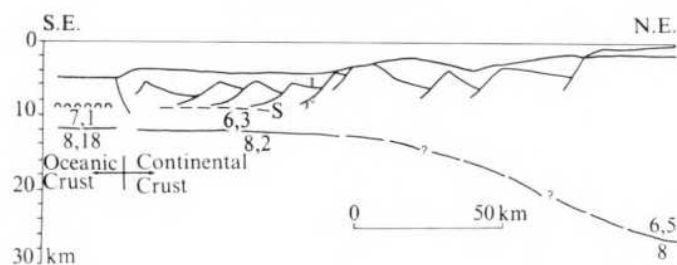


Fig. 6 Absolute amount of post-rifting subsidence of N. Biscay margin from the shelf to the oceanic crust. Upper dotted line, simplified topography of the rift at Aptian time; lower dotted line, present depth of Aptian corrected for loading; solid line, amount of subsidence.

subsidence rate cannot be defined with precision, however, because palaeontologically determined depths become increasingly inaccurate for increasing water depths and age. Nevertheless, data⁴ do not contradict an exponential decrease in subsidence. The subsidence of the thinned continental crust adjacent to the continent-ocean boundary is greatest and most similar to the ocean crust, whereas that of the thick crust beneath the shelf is least and most different. The magnitude of the subsidence is thus clearly related to crustal thickness. Change of slope on the subsidence against distance curve at 150 km (Fig. 6) may reflect abrupt changes in crustal thickness. The curve also substantiates the coupling of the continental and oceanic crust because subsidence of the oceanic crust is approximately equal to the continental oceanic crust.

Unfortunately, we cannot address the role of the ductile layer, Moho and Upper Mantle on the subsidence during and after rifting. Their nature and position may well have been different during and after rifting. Nonetheless, spatial variations in the post-rift subsidence of the passive margins of Biscay are at least a partial function of crustal attenuation produced by thinning of the ductile layer during rifting.

Fig. 5 Schematic crustal sections through the N. Biscay margin (data refs 2, 19).



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A new Thy-1 alloantigen as a temporal marker of T lymphocyte differentiation

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A new Thy-1 alloantigenic specificity is defined in the mouse. Antigen expression is restricted to 'mature' and memory T cells, with either helper or killer activity.

THE importance of lymphocyte differentiation antigens in defining the cellular heterogeneity of the immune system is now well established; functionally distinct lymphocyte populations exhibit a unique surface antigen phenotype, and may be enriched or depleted with specific antibody¹. In the mouse, the initial classification of lymphocytes as B or T cells was based on surface expression of immunoglobulin or the Thy-1 alloantigen^{2,3}. Subpopulations of T cells have since been defined. These are distinguished by their differential expression of the alloantigens Ly-1, Ly-2 and Ly-3 (refs 4–6). Studies by Cantor and Boyse with the Ly markers indicate two independent pathways for the differentiation of T lymphocytes^{7,8}: thymocytes and 'precursor' T cells with a Ly-1⁺ 2⁺ 3⁺ surface phenotype generate either helper cells (Ly-1⁺ 2⁺ 3⁺) or suppressor/killer cells (Ly-1⁺ 2⁺ 3⁺), commitment to either differentiation pathway preceding exposure to antigen. However, virgin and memory T lymphocytes cannot be distinguished by means of these markers.

Evidence is presented here for a new Thy-1 alloantigen which may be used to distinguish virgin and memory T cells. The antigen is absent from neonatal T cells but present on 50% of the adult T cell population. Functional tests indicate that this new specificity is restricted to 'mature' and memory T cells, with either helper or killer activity.

Xenoantisera to Thy-1

Experiments with xenoantisera to murine tissues provided the first, fortuitous indication that Thy-1 included an hitherto unrecognised specificity. Rats and AKR mice both express the Thy-1.1 alloantigen on brain and thymus, and reciprocal immunisation should only elicit antibody to xenogeneic determinants. It was unexpected to find that rat antiserum to AKR brain defined a subpopulation of T lymphocytes (Fig. 1).

Xenoantiserum dilutions of log₃ 1–3 and C' (guinea pig complement) lysed a constant number of cells in AKR thymus (75–80%), lymph node (30–35%) and spleen (18–20%),

without affecting bone marrow cells (<4%). (Values are also included in Fig. 1 for lysis by Thy-1.1 alloantibody and C'.) Cytotoxicity was removed by absorption with AKR brain, but not rat brain or thymus, thereby excluding autoantibody activity.

Identical values were obtained with lymphoid tissues of Thy-1.2-positive mice: CBA, C57BL/6, BALB/c, LPJ, C3H/he and A strains. However, spleen cells from outbred, athymic *nu/nu* mice were not lysed (<3%), suggesting specificity for a subpopulation of T lymphocytes (about 50% of Thy-1⁺ cells in spleen and lymph node, Fig. 1). This was confirmed in experiments with mitogen-treated cultures of CBA spleen. Xenoantiserum (1/3 to 1/9 dilution) and C' lysed concanavalin A-induced T lymphoblasts (35–50%) and phytohaemagglutinin A-induced T lymphoblasts (18–25%) but not lipopolysaccharide-induced B lymphoblasts (<4%) at the peak of the mitogenic response (48–72 h).

The number of peripheral lymphocytes lysed by xenoantiserum and C' showed a striking change with age. There was no appreciable lysis by xenoantiserum during the first 2 weeks of

Table 1 Indirect immunofluorescent staining of CBA spleen cells with xenoantibody or alloantibody after capping of surface immunoglobulin

	Fluorescent cells (% ring staining)
Anti-BAT	25
Anti-Thy-1.2	41
Anti-Thy-1.2 ab	22
FITC-anti-mouse IgG	4
FITC-anti-rat IgG	3

CBA spleen lymphocytes ($5-10 \times 10^7$; 3 ml) were incubated with fluorescein isothiocyanate (FITC)-conjugated rabbit anti-mouse IgG (Miles; 1/20 dilution) for 2 h at 37°C (minimum time for complete capping) and washed twice with buffered saline. Aliquots ($2-4 \times 10^6$; 100 μ l) were then incubated with an equal volume of anti-BAT (1/3–1/12 dilution) or anti-Thy-1.2 ab (1/10–1/30 dilution) or anti-Thy-1.2 (1/10–1/30 dilution) for 30 min at 3°C, washed and incubated for 30 min at 3°C with 50 μ l of FITC-conjugated rabbit anti-rat IgG or FITC-conjugated rabbit anti-mouse IgG. Cells were washed and examined with a Leitz Ortholux fluorescence microscope.

life (Fig. 2; <4%). Moreover, absorption of antiserum with newborn CBA spleen (1: 1, v/v; 2 h at 3 °C) did not alter the cytotoxic titre for adult spleen or lymph node, thus indicating absence of a particular cell population from the neonate. However, the adult number of T cells were lysed by Thy-1 alloantibody within a day of birth. Henceforth, the antigen(s) recognised by rat anti-AKR brain sera will be referred to as 'BAT' (brain-associated thymus antigen).

Effect of thymectomy

The neonatal mouse is immunologically incompetent and surgical removal of the thymus drastically, and permanently, impairs homograft immunity, whereas thymectomy at 3 weeks has little effect^{9,10}. However, the proportion of Thy-1⁺ cells in peripheral

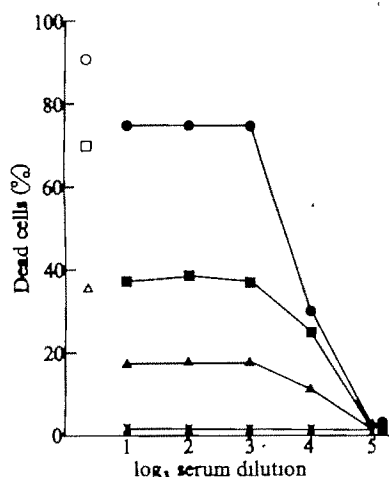


Fig. 1 Cytotoxic titre of rat anti-AKR brain serum and C' for AKR lymphoid cells: thymus (●), lymph node (■), spleen (▲) and bone marrow (×). Values are also included for lysis of thymus (○), lymph node (□) and spleen (△) by anti-Thy-1.1 (1/16 dilution: Searle Diagnostic) and C'. Rats, either outbred Wistar or inbred PVG and August strains, received five weekly intraperitoneal injections of AKR brain homogenate (1:1, v/v; phosphate-buffered saline, PBS). Sera from 6–8 rats were pooled, heat-inactivated and absorbed with mouse liver homogenate to remove species antibody (1:1, v/v; 2 h at 3 °C). Viable lymphocytes (>98%) were obtained by Ficoll-Hypaque centrifugation of tissue cell suspensions. In the cytotoxic assay, equal volumes of cells (2.5×10^6 ; 50 μ l), xenoantiserum and C' (absorbed with Agarose) were incubated at 37 °C for 30 min and washed with PBS (2 ml). A two-step incubation was used for anti-Thy-1.1 to avoid the anti-C' activity of mouse serum. Cell viability was estimated by dye exclusion using eosin (0.1%, w/v). Control values for normal rat serum (absorbed with mouse liver) and C' were <4% dead cells.

lymphoid tissues is identical in the neonate and adult (ref. 11, Fig. 4a), perhaps indicating further, thymus-dependent, maturation of the peripheral T cell in the first 2 weeks of life.

The percentage of cells reacting with xenoantibody showed an abrupt increase during this time (Fig. 2). An attempt was made to determine whether thymectomy affected the appearance of the BAT⁺ cell population. CBA mice were thymectomised at 3 d or at 3 weeks, and killed at weekly intervals thereafter; pooled spleen cells from six or more donors were treated with xenoantibody or anti-Thy-1.2 and C'. Neonatal thymectomy delayed, but did not prevent the appearance of BAT⁺ cells (Fig. 3a). However, adult thymectomy produced a reciprocal decrease in the Thy-1⁺ cells and increase in BAT⁺ cells, so that at 5 weeks post-thymectomy these populations were numerically equivalent (Fig. 3b).

Dual specificity of Thy-1 alloantisera

The existence of a new murine antigen (BAT) unrelated to Thy-1, but restricted to brain and the T cell series seems unlikely. Alternatively, Thy-1 itself might define two serological determinants differing in temporal expression: 'conventional'

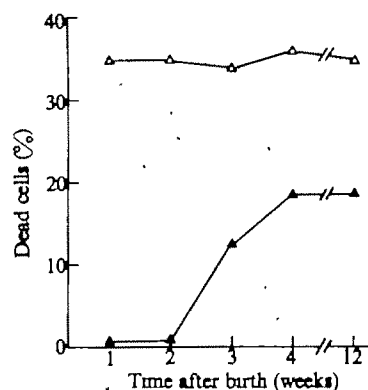


Fig. 2 Cytolytic effect of rat anti-AKR brain serum (▲, 1/6 dilution) or anti-Thy-1.2 (△, 1/16 dilution) and C' on CBA spleen cells of different ages.

Thy-1 present on neonatal and adult T cells, and a second specificity restricted to the adult. Alloantisera might then be expected to contain dual specificities, distinguished only by their reactivity with neonatal T cells.

To test this possibility, alloantisera (C3H anti-AKR, or AKR anti-C3H) were absorbed with 2–3-d-old spleens from either AKR or CBA mice and examined for residual activity. Absorption of Thy-1.2 (Fig. 4b) or Thy-1.1 (Fig. 4c) alloantisera had no effect on the cytotoxic titre for adult tissues but removed all activity for neonatal spleen. The age of the spleen cells used for absorption was critical; 8–10-d-old donor cells removed all cytolytic activity.

Plateau values for the percentage of cells lysed (thymus, spleen and lymph node) were obtained with alloantiserum dilutions of $\log_2 2-6$ (Fig. 4a; cytotoxic index and titre of anti-Thy-1.1 identical, data not presented). Absorption reduced these values for each tissue to give profiles strikingly similar to those obtained with xenoantisera (Fig. 1); thymus 80%, spleen 18–20%, lymph node 30–35%. Adult AKR thymus (Fig. 4c; 50%) was the obvious exception. These results suggested that xenoantisera and absorbed alloantisera were recognising the same cell population. To confirm this, cytotoxic values were obtained after consecutive addition of alloantisera and C', and xenoantiserum and C', to CBA or AKR spleen, or lymph node cells. The number of cells lysed remained unaltered after addition of xenoantiserum.

Murine alloantisera are frequently contaminated with viral antibody, leading to some confusion over serological specificity. It might be argued that the lymphocytotoxic antibody, removed by absorption with neonatal spleen, was directed against viral determinants, residual activity being *bona fide* Thy-1 antibody (Fig. 4b, c). This argument presupposes the absence of T cells in neonatal spleen; this is unlikely, as monoclonal Thy-1.2 antibody and C' lysed 30–35% CBA spleen cells from both 3-d-old and adult donors. Also, xenoantiserum and absorbed Thy-1.1 alloantibody lysed an equivalent number of spleen cells (20%) in

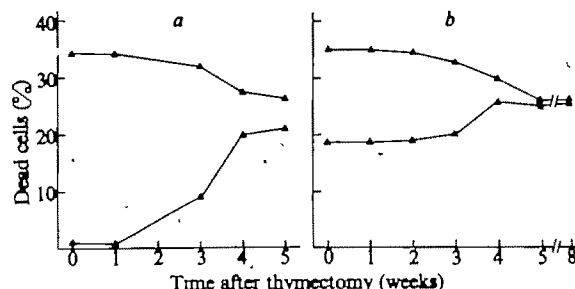


Fig. 3 Effect of neonatal (a) or adult (b) thymectomy on the number of CBA spleen cells lysed by rat anti-AKR brain serum (▲, 1/6 dilution) or alloanti-Thy-1.2 serum (△, 1/16 dilution) and C'.

congenetic A strain mice, where Thy-1.1 occurred on a different genetic background.

Lymphocytotoxicity compared with immunofluorescence

Lymphocytotoxic tests often give spurious results due to the presence of non-complement-fixing antibodies. Failure to lyse all T cells with xenoantibody or absorbed alloantibody might be an artefact of C fixation, and misinterpreted as a new serological specificity. It was necessary, therefore, to confirm these estimates by a different technique. Indirect immunofluorescence is a reliable method of detecting cell-bound antibody, although staining of lymphocytes with alloantibody or rat antibody includes a high background staining of immunoglobulin-bearing B cells. This background may be reduced by an initial 'capping' of B cells with antiglobulin.

Reasonable agreement was obtained by indirect immunofluorescence and no more than 20% of spleen cells bound xenoantibody or absorbed alloantibody. Both tests, therefore indicate that the sera are specific for a subpopulation of T cells.

Significance of a second Thy-1 alloantigen

We have shown that Thy-1 alloantisera contain two distinct antibody specificities, distinguished by absorption with neonatal T cells, whereas xenoantisera recognise a Thy-1 determinant restricted to the adult T cell.

The *Thy-1* locus, *Thy-1^a* and *Thy-1^b* alleles, is on chromosome 9 of the mouse, and there is no evidence of polymorphism¹². Biochemical studies in the rat, however, indicate molecular heterogeneity of Thy-1. Williams *et al.*, using lectin-affinity chromatography, have resolved Thy-1 from rat thymocytes into two components with molecular weights of 25,000 and 27,000 (ref. 13). Differences in lectin affinity were attributed to the carbohydrate moiety rather than the polypeptide. It is reasonable to assume that the dual specificities, defined here, also reflect post-translational modification of Thy-1, possibly by glycosylation. It may be anticipated that monoclonal Thy-1 antibodies, produced by hybridoma cell lines, will be specific for either 'conventional Thy-1' or the 'alternative Thy-1' defined here.

We describe below our attempts to attribute a function to the T cell population reacting with xenoantibody (anti-BAT), and the alloantibody, absorbed with neonatal spleen cells (anti-Thy-1 ab).

Phenotype of helper T cells

Helper T cell function was assayed by adoptive cell transfer. Spleen cells from normal or adult-thymectomised mice were transferred, together with sheep red blood cells (SRBC) as

immunogen, into lethally irradiated syngeneic mice; recipients were assayed for anti-SRBC plaque-forming cells (PFC) after 6 d.

The anti-SRBC response requires helper T cell cooperation. Pretreatment of donor lymphocytes with anti-Thy-1 and C abolished the development of direct and indirect PFC (Table 2, group 1). However, removal of >50% of T cells with anti-BAT or anti-Thy-1 ab and C had no effect on the primary anti-SRBC response, and even elevated the number of indirect PFC. Spleen cells transferred from mice, adult-thymectomised 4 months previously, developed a reduced number of PFC in irradiated hosts (Table 2, group 2). Here, in contrast, the number of PFC was reduced even further by anti-BAT or anti-Thy-1 ab and C. Cells from normal mice, primed 4 months earlier with SRBC, developed an increased number of PFC (Table 2, group 3). Treatment with anti-BAT and C now reduced the number of indirect PFC but had no effect on direct PFC. These results indicate that helper cells in the primary response to SRBC are BAT⁺ Thy-1 ab⁻, but BAT⁺ Thy-1 ab⁺ in thymectomised mice; in a secondary response, only a proportion of helper cells are BAT⁺.

The increased number of indirect PFC from the virgin donors treated with antibody and C may be due to elimination of suppressor T cells, but as we have no experimental system to generate or estimate such, this cannot be confirmed.

Phenotype of killer T cells and their precursors

Two experimental protocols were used either an adoptive cell transfer of CBA lymphocytes into lethally irradiated F₁ (CBA × DBA/2) recipients (*H-2^{k/d}*) to generate alloreactive (*H-2^d*) killer cells, or challenge of normal or adult-thymectomised, CBA mice with allogeneic tumour. Killer activity was assayed by the chromium release method using the P815Y tumour as a target.

Pretreatment of donor lymphocytes, or recipient F₁ cells at the time of assay, with anti-BAT or anti-Thy-1 ab and C had no effect on killer activity, indicating that neither precursor nor effector cells expressed the antigen(s) (Table 3, group 1). However, contradictory results were obtained in the second series of experiments (Table 3, group 2). Normal CBA mice were immunised with allogeneic tumour and killer activity was assayed at various times thereafter. Optimum killer activity was generated at 12 d; between 12 and 18 d ⁵¹Cr release was unaffected by antibody and C, whereas at 22 or 35 d values were reduced to base line.

This finding was constantly reproducible. Killer cells became susceptible to cytotoxic treatment with antibody and C at 20–23 d following immunisation. In CBA mice thymectomised as adults and challenged with allogeneic tumour 5 weeks later,

Fig. 4 Cytotoxic titre of Thy-1 alloantisera before (a, anti-Thy-1.2) and after (b, anti-Thy-1.2; c, anti-Thy-1.1) absorption with neonatal spleen. Alloantisera (Searle Diagnostic) were absorbed (1/4 dilution) with an equal volume of packed neonatal spleen cells, either CBA or AKR (1:1, v/v; 2 h at 3 °C) and assayed for residual cytotoxic activity: adult thymus (○), lymph node (□), spleen (△), neonatal spleen (Δ).

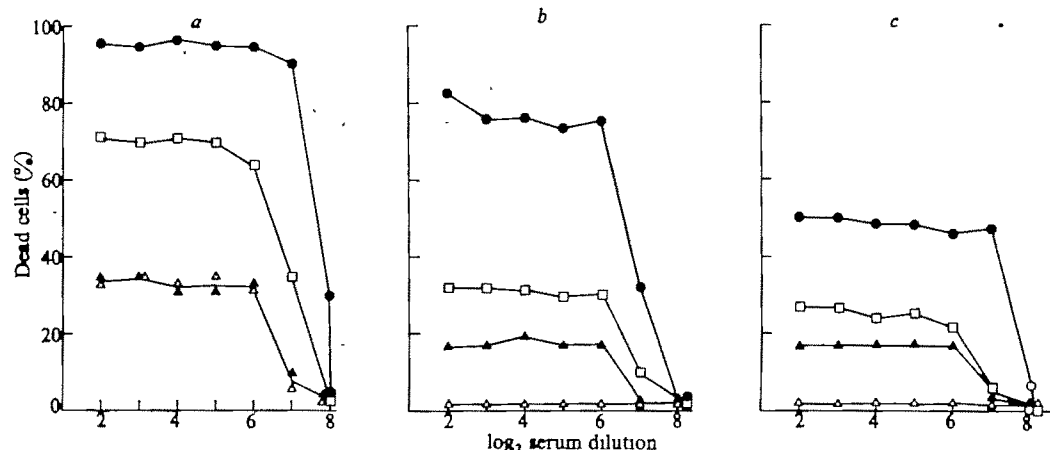


Table 2 Effect of xenoantibody or alloantibody and C' on the generation of a primary and secondary antibody response to SRBC in irradiated hosts

Group	Cell source	Direct PFC per 10 ⁶ cells	Indirect PFC per 10 ⁶ cells
1	Spleens of virgin CBA mice, treated with*:		
	NRS+C'(<2%)†‡	1,193 (898–1,439)	1,781 (1,635–2,126)
	Anti-BAT+C'(20%)†	1,524 (1,111–2,083)	3,016 (2,142–3,640)
	Anti-Thy-1.2+C'(32%)†	138 (90–221)	293 (192–442)
	Anti-Thy-1.2 ab+C'(18%)†	1,030 (972–1,094)	1,736 (1,370–2,187)
2	Spleens of adult-thymectomised CBA mice, treated with*:		
	NRS+C'(<2%)†	296 (257–358)	920 (708–1160)
	Anti-BAT+C'(24%)†	127 (107–145)	205 (187–237)
	Anti-Thy-1.2 ab+C'(21%)†	81 (64–116)	237 (209–262)
3	Spleens of primed CBA mice, treated with*:		
	NRS+C'(<1%)†	3,299 (2,931–3,742)	28,666 (25,000–33,250)
	Anti-BAT+C'(18%)†	3,517 (3,302–3,716)	14,020 (13,096–14,721)
	Anti-Thy-1.2+C'(34%)†	673 (531–789)	5,035 (3,986–5,580)

Spleen cells from six or more mice were pooled and assayed for PFC by the slide method, as described by Dresser¹⁹. Values for PFC per 10⁶ spleen cells represent the mean and range for six individual determinations.

* Viable lymphocytes were obtained by Ficoll-Hypaque centrifugation of CBA spleen cells. An equal volume of cells (4×10^8 ; 1 ml), C' and normal rat serum (NRS, 1/3 dilution), or anti-BAT (1/3 dilution), or anti-Thy-1.2 (1/8 dilution), or anti-Thy-1.2 ab (1/8 dilution) were incubated for 30 min at 37 °C. Lymphocytes (2×10^7 ; 0.3 ml) and SRBC (10^8) were injected intravenously into irradiated (900 R) CBA mice. Recipient mice were killed after 6 d.

† Number of cells lysed by antiserum and C' before adoptive transfer.

killer activity was always eliminated by anti-C' antibody (Table 3, group 3).

CBA mice are exceptional; their killer cells express a Ly-1⁺2⁺3⁺ phenotype whereas most other strains, including C57BL/6, are Ly-1⁺2⁺3⁺ (ref. 14). Therefore, the experiments were repeated with C57BL/6 mice (Table 3, group 4) with similar results: killer activity was eliminated by anti-BAT and C' at 22 d but not at 12 d. One may conclude that pre-killer and killer T cells are BAT⁺, Thy-1 ab⁺, whereas memory killer cells are BAT⁺ Thy-1 ab⁺.

A two-compartment model of antigen expression

Figure 5 schematically summarises our results. Two compartments for the peripheral T cell population are defined antigenically, BAT⁺ Thy-1 ab⁺ and BAT⁺ Thy-1 ab⁺. These antigens are distinct from any other lymphocyte differentiation antigen so far described in that they are markers of time not function. This may allow the estimation of transit time between

two cell compartments: virgin (or primary effector) cells and mature (or memory) cells. Lymphocytes leaving the thymus are BAT⁺ Thy-1 ab⁺. Removal of the source of precursor cells (by adult thymectomy) produces a majority of BAT⁺ Thy-1 ab⁺ peripheral T cells within 30–35 d (Fig. 3, Tables 2, 3).

The time required to generate memory killer T cells may also be estimated. After immunising normal CBA mice with allogeneic tumour, T killer cells are initially BAT⁺ Thy-1 ab⁺ (Table 3; 12–18 d) but within 20–23 d killer activity is present in the BAT⁺ Thy-1 ab⁺ population. In adult-thymectomised mice, killer cells are BAT⁺ Thy-1 ab⁺ at all times.

Raff and Cantor have proposed two major categories of peripheral T cells¹². The T₁–T₂ concept was a useful working model which gave some rationale to earlier reports of synergy between different Thy-1⁺ cell populations^{16,17}. T₁ cells were defined as short-lived, non-recirculating lymphocytes that were susceptible to adult thymectomy. T₂ cells were long-lived, recirculating lymphocytes; they were resistant to adult thymectomy but highly sensitive to the *in vivo* effect of anti-lymphocyte serum. This T₁–T₂ classification is somewhat overshadowed by

Table 3 Effect of xenoantibody or alloantibody and C' on the generation and activity of alloreactive, killer T cells

Group	Cell source	Assay time (d)	⁵¹ Cr release (%)§ NRS+C'	Anti-BAT+C'	Anti-Thy-1.2+C'	Anti-Thy-1.2ab+C'
1.	Adoptive transfer (CBA → CBA/DBA-2)					
	Pre-killer cells*	7	57	54	11	ND
	Killer cells†	7	53	60	8	57
2.	Allogeneic response: normal CBA mice‡	12	49	53	12	49
		14	68	64	12	66
		18	75	66	10	63
		22	70	11	8	10
		35	62	12	11	9
3.	Allogeneic response: adult thymectomised mice‡	12	52	13	9	13
		22	60	10	10	12
4.	Allogeneic response: normal C57BL/6 mice‡	12	78	71	13	ND
		22	68	10	10	ND

* Viable lymphocytes, obtained by Ficoll-Hypaque centrifugation, were treated with antiserum and C', as in Table 1. Donor lymphocytes (2×10^7 ; 0.3 ml) were injected intraperitoneally into irradiated (900 R) F₁ hosts.

† Irradiated F₁ recipients were killed at 7 d; spleen cells were treated with NRS, or antiserum, and C' and assayed for killer activity.

‡ Mice were injected intraperitoneally with 10⁷ P815Y tumour cells and killed at various time intervals (12–35 d). Ficoll-Hypaque-purified spleen cells were treated with NRS, or antiserum, and C' before assaying for killer activity.

§ Spleen cells (2×10^7 /ml) were incubated with ⁵¹Cr-labelled tumour (2×10^5 /ml) for 5 h at 37 °C, centrifuged, and radioactivity in the supernatant determined. Values are given for % of total radioactivity in the supernatant; complete target lysis is equivalent to 75–80% release. Spontaneous release of ⁵¹Cr, in the absence of spleen cells, was 8–12%. ND, not determined.

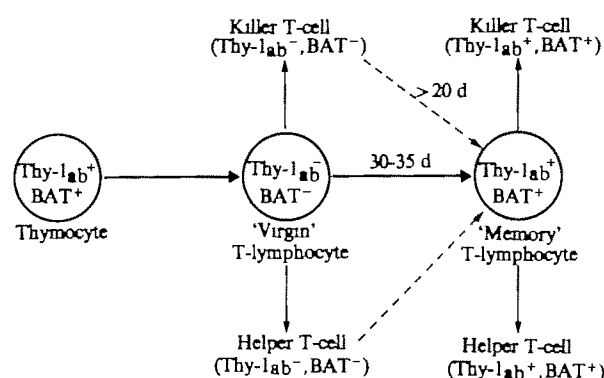


Fig. 5 A scheme of differential antigen expression by murine lymphoid cells.

recent studies with the Ly alloantigens^{7,8}, however, in functional terms, the T_2 population includes both $Ly-1^+$ helper and $Ly-2^+3^+$ suppressor/killer cells, and T_1 represents the precursor population of $Ly-1^+2^+3^+$ cells.

Some analogies may be made between $Ly-1^+2^+3^+$ (or T_1) lymphocytes and the $BAT^- Thy-1 ab^-$ population (Fig. 5). In the neonate, T cells are exclusively $Ly-1^+2^+3^+$ and $BAT^- Thy-1 ab^-$ (Figs 2, 4b, c) and both account for 50% of the adult population (Figs 1, 4b, c); the numbers of $Ly-1^+2^+3^+$ cells and $BAT^- Thy-1 ab^-$ cells (Fig. 3b, Tables 2, 3) are decreased after adult thymectomy⁷. The $Ly-1^+2^+3^+$ phenotype, however, defines a precursor population that differentiates further to either helper ($Ly-1^+2^+3^-$) or killer ($Ly-1^+2^+3^+$) cells^{7,8}. The $BAT^- Thy-1 ab^-$ population includes precursor cells and primary effector cells, and antigen expression reflects time not function.

The above model (Fig. 5) presents an obvious paradox. In the adult mouse, equal numbers of peripheral T cells are $BAT^- Thy-1 ab^-$ and $BAT^+ Thy-1 ab^+$; both populations can generate killer cells and helper cells (Tables 2, 3). However, the effector cells in a primary immune response are exclusively $BAT^- Thy-1 ab^-$. Following adult thymectomy, effector cells are $BAT^+ Thy-1 ab^+$ and helper function is somewhat reduced. These findings imply that, during a primary immune response, there is

a selective recruitment of 'young' T cells which have just left the thymus.

Certain reservations must be made concerning helper function. Further experiments are required with limiting numbers of T cells, for both the primary and secondary antibody response of irradiated hosts, to demonstrate conclusively that most helper cells are $BAT^- Thy-1 ab^-$.

If this scheme is essentially correct and there is recruitment of young T cells, several predictions can be made and tested experimentally. For example, the ageing mouse shows a progressive decline in thymic function and a reduced antibody response¹⁸. The age-dependent changes in humoral immunity may mirror, or even be a consequence of, altered ratios in the two T cell compartments, in which case, old mice might resemble adult-thymectomised mice.

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A suppressor mutation in the nematode acting on specific alleles of many genes

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A suppressor mutation has been isolated in Caenorhabditis elegans through reversion analysis of a muscle-defective mutant. The suppressor mutation acts on specific alleles of at least six genes and in one case we have been able to show that it partially restores functional gene product to a mutant otherwise lacking that product. These and other features of the suppressor suggest that it acts at some step in information transfer, perhaps through mechanisms similar to those described previously in microorganisms.

In intergenic suppression, the effects of a mutation in one gene are alleviated by mutations in other genes. Many different suppressor genes have been found in microorganisms, where restoration of the wild phenotype is achieved by a variety of mechanisms. Some suppressor genes compensate for the ori-

ginal mutation by affecting subunit interactions in macromolecular assemblies¹⁻⁴. Others act more indirectly by providing alternative biochemical pathways⁵⁻⁷ or by changing the physiological balance of the cell^{8,9}. A particularly important class is that of informational suppressors (for review see refs 10-13), which alter the fidelity of translation of mutant messages to allow the production of functional gene product, by changes in either transfer RNA¹⁴⁻¹⁹ or some other component of the translation machinery¹¹.

The identification and analysis of suppressor genes in microorganisms have provided an approach to problems that were not readily accessible with other methods of study. In multicellular organisms, the analysis of mutants and their suppressors might lead to the identification of the primary products of genes participating in developmental pathways and thereby provide some hint of the molecular interactions involved in this complex

Table 1 Summary of the results of four 3-factor crosses used to locate *sup-5** in LGIII

Cross	Parental genotype	Number of each recombinant genotype obtained	
1	<i>e1464</i> + + + <i>unc-69</i> <i>dpy-18</i>	<i>e1464</i> + <i>dpy-18</i> 10	+ + <i>dpy-18</i> 0
2	<i>e1464</i> + <i>dpy-18</i> + <i>unc-69</i> +	+ <i>unc-69</i> <i>dpy-18</i> 12	+ + <i>dpy-18</i> 8
3	<i>e1464</i> + + + <i>unc-32</i> <i>dpy-18</i>	<i>e1464</i> + <i>dpy-18</i> 21	+ + <i>dpy-18</i> 0
4	+ + <i>e1464</i> <i>lon-1</i> <i>dpy-19</i> +	<i>lon-1</i> + <i>e1464</i> 6	<i>lon-1</i> + + 0

To establish linkage, 20 suppressed animals were picked from among the progeny of hermaphrodites of the genotype *unc-15/+*; *m/+*; *sup-5*+, with the marker (*m*) being tested *trans* to the suppressor. The progeny of these suppressed animals were inspected to confirm that the parent was homozygous for the suppressor and for the presence of the marker. In all cases, except when markers from LGIII were used, the progeny of approximately 2/3rds of the parents included *m* animals. With markers from LGIII, many fewer parents contained the marker mutation. The mutant alleles used in the crosses were *unc-69*(*e587*), *dpy-18*(*e364*), *dpy-19*(*e1259*) and *lon-1*(*e185*)²⁶. For the 3-factor crosses, appropriate double mutants were constructed and mated with males carrying the third mutation to obtain the hermaphrodites listed under parental genotype. Among the progeny, animals with a recombinant phenotype were selected. In crosses 1, 2 and 3, dumpy animals were picked and progeny of these animals examined to determine the recombinant genotype, taking advantage of the observation that *dpy-18*(*e364*) *sup-5*(*e1464*)/*dpy-18*(*e364*) + animals are partially suppressed but still obviously dumpy and that *dpy-18*(*e364*) *sup-5*(*e1464*) homozygotes are nearly fully wild. From cross 4, long recombinants were selected, as *dpy-19* is epistatic to *lon-1*. Strains homozygous for the recombinant chromosome were obtained, and the presence of the suppressor was ascertained by testing with *unc-15*(*e1214*).

* Gene names in *C. elegans* consist of an italicised 3-letter abbreviation for a general phenotypic character, (for example, *unc* for uncoordinated, *dpy* for dumpy and *sup* for suppressor) separated by a dash from an italicised number to denote the specific gene, for example, *sup-5*. When appropriate an italicised roman numeral may follow, to indicate the linkage group of the gene, for example, *sup-5* III. Mutation names consist of 1 or 2 lower case italicised letters (used to indicate the laboratory of origin) followed by an italicised number, for example, *e1464*. *e* denotes mutations from the Cambridge collection and *st* denotes mutations from the St Louis collection. When gene and mutation names are used together the gene name is followed by the mutation name in parentheses, for example, *sup-5*(*e1464*) III. Strain names consist of 1 or 2 non-italicised upper case letters (indicating the laboratory of origin) followed by a number, for example, E1464. For most single mutants, strain and mutation names have the same letter and number but differ in typecase and by typeface, for example, the strain E1464 and the mutation *e1464*. Strains with multiple mutations are designated by the 1- or 2-letter laboratory prefix, a dash and a single upper case letter indicating the type of multiple mutant and number. Thus R is used to denote revertant strains, for example B-R215.

process. Especially useful would be a set of informational suppressors, which could then be used to study a wide variety of genes. Although suppressor mutations have been identified in *Drosophila melanogaster* (see ref. 20 for list), the mechanism involved is only partially understood²¹⁻²⁷.

For several years we have been studying mutants with abnormal muscle in the nematode *Caenorhabditis elegans* to identify genes involved in muscle assembly. Of the 13 genes now known to affect muscle structure (R.H.W., unpublished) the products of two are now known: *unc-15* I specifies paramyosin²⁹ and *unc-54* I specifies a major heavy chain of myosin³⁰⁻³³ (see footnote to Table 1 for nomenclature). At both loci we have isolated mutants that produce altered products, as well as null mutants in which none of the relevant polypeptide product can be detected²⁹⁻³². Mutants in these genes are particularly suitable for studies of suppression, for a few animals with even marginally enhanced motility can be easily detected against the background of the original paralysed mutant. Effects of suppression on muscle structure can be screened by polarised light microscopy, and biochemical analysis of the consequences of suppression can be readily carried out with the null mutants.

Reversion analysis of a large set of mutants with severely defective movement has already been carried out and some suppressor genes have been identified (refs 34, 35 and S.B., unpublished). In none of these cases is the molecular mechanism of compensation understood. In particular, Riddle and S.B.³⁴ have characterised a suppressor (*sup-3* V) that partially reverses the phenotypic effects of many alleles of *unc-15*, *unc-54* and *unc-87* I, another gene affecting muscle (R.H.W., unpublished). This and other evidence suggest that *sup-3* probably acts indirectly.

We report here the identification of a different suppressor (*sup-5* III) isolated initially in reversion studies of E1214, a mutant of *unc-15* that is very paralysed, is fertile, but does not lay eggs, and contains no detectable paramyosin²⁹. The suppressor is allele-specific but not gene-restricted, suppressing

only some alleles in several different genes. It also has the important property of partially restoring paramyosin in animals homozygous for *e1214*. These observations suggest that *sup-5*(*e1464*) III might be an informational suppressor.

Isolation and preliminary genetic characterisation of the suppressed strain R215

Approximately 10⁵ E1214 animals, treated with 0.05 M ethyl-methane sulphonate³⁵, were deposited on one side of 24 10-cm Petri plates. No full revertants appeared over the next three generations, but several plates contained animals with possibly improved movement. Most of these were later discarded but one strain that showed distinctively improved mobility was retained. Adults of this strain were larger than the E1214 strain and laid eggs, and the uniformity of the phenotype in the progeny indicated that the revertant was homozygous. F₂ progeny from a cross of the revertant with wild-type males showed that the revertant contained the original *e1214* mutation as well as an unlinked suppressor mutation (*C. elegans* normally reproduces as a self-fertilising hermaphrodite. It is diploid, with five autosomal linkage groups (I-V) and a sex linkage group (X). Males, which are hemizygous for the sex chromosome, are used to transfer genetic material. Heterozygous hermaphrodites can be allowed to self-fertilise to obtain homozygous animals.) From this cross a homozygous suppressed strain was re-isolated and propagated as the strain E-R213. To remove extraneous mutations, this strain was first crossed with *dpy-5* *unc-15*(*e1214*)/+ + males to obtain a dumpy suppressed strain which was in turn crossed with *unc-15*(*e1214*)/+ males to remove the dumpy marker. This yielded the strain E-R215 which was homozygous for both *e1214* and the suppressor allele, designated *e1464*. E-R215 grows better than E-R213, but motility and other characteristics do not differ appreciably.

Mapping the suppressor, *e1464*, in linkage group III

Location of the suppressor on linkage group III (LGIII) was determined initially from 2-factor crosses in a homozygous *e1214* background using general genetic methods described previously³⁶. An estimate of 7 map units between *dpy-18 III* and the suppressor was obtained, and results with *dpy-19 III*, *sma-2 III* and *lon-1 III* indicated that *e1464* was located less than 4 map units from these markers. As no previously mapped suppressors were in this region, *e1464* was assigned to a new gene *sup-5 III*. A strain containing the suppressor in a wild-type background, E1464, was obtained by elimination of the *lon-1* marker from a *lon-1 +/+ e1464* animal.

A male stock of the E1464 strain was generated from spontaneously occurring males to facilitate the transfer of the mutation in further genetic manipulations. Several 3-factor crosses were carried out to position the suppressor more precisely (Table 1). In progeny of hermaphrodites of the genotype *sup-5(e1464) +/+ unc-69 dpy-18* in cross 1, all the dumpy recombinants contained the suppressor, indicating that *sup-5(e1464)* lies to the left of *unc-69* or very close to it. From cross 2, only 12 of 20 dumpy recombinants contained the *trans* mutation *unc-69*, clearly placing *sup-5* to the left of *unc-69*. A third cross was carried out with *e1464 trans* to *dpy-18* and *unc-32*, a gene 3% to the left of *unc-69*. All dumpy recombinants contained the suppressor. A left-hand limit was established in cross 4, where *sup-5* was shown to lie to the right of *lon-1*. Crosses 3 and 4 place *sup-5* very close to *dpy-19* and *unc-32*, two genes that are themselves only 0.1% apart (Riddle, personal communication).

Phenotype of E1464

The E1464 strain moves normally, and its muscle structure as observed with polarised light microscopy²⁹ is indistinguishable from wild type. However, the strain shows some differences from wild type. Sterile animals are produced frequently, particularly when the strain is grown at 15 °C instead of room temperature (22 °C). Occasionally, dead early larvae and other abnormal forms have been noted. The generation time is increased to 7.5 d at 15 °C from the normal wild-type time of 5.5 d. Male stocks have been very difficult to maintain. Doubles of *e1464* with the linked markers *e364* and *e185* exhibit similar characteristics. This fact, together with the several crosses undergone in the construction of the E1464 strain makes it probable that these properties are due to the suppressor mutation itself.

Range of suppression by *e1464*

The *e1464* suppressor has been combined with a large number of different mutants of many genes to test for suppression. While mapping *e1464*, the phenotype of many mutants was found to be unaffected by *e1464*. These include the dumpy mutants *dpy-5(e61) I*, *dpy-10(e128) II*, *dpy-18(e189) III*, *dpy-13(e184) IV* and *dpy-11(e224) V*; the uncoordinated mutant *unc-32(e189) III*; the long mutant *lon-1(e185) III*; and the small mutant

sma-2(e502) III (ref. 36). However, *dpy-18(e364) III* gave anomalous results in initial mapping experiments, and subsequently it was shown that *e364* is suppressed by *e1464*. In strains homozygous for both *e364* and *e1464*, animals are nearly wild type in length and diameter when grown at 15 °C (Table 2).

Additional double mutants have been constructed to test the specificity of *e1464*-mediated suppression. In the *unc-15* complementation group, the mutant allele, *e73*, which results in a dysfunctional paramyosin, is not suppressed even though the null allele, *e1214*, is.

unc-54 mutants have also been studied, as the gene product is known to be a myosin heavy chain³⁰⁻³³. More than 30 independent mutations are available, and these have been classified into two groups; mutants of the larger class contain no detectable *unc-54* myosin, whereas those of the other contain approximately normal levels of gene product³¹. This second class is assumed to have altered product and, in fact, one mutant, E675,

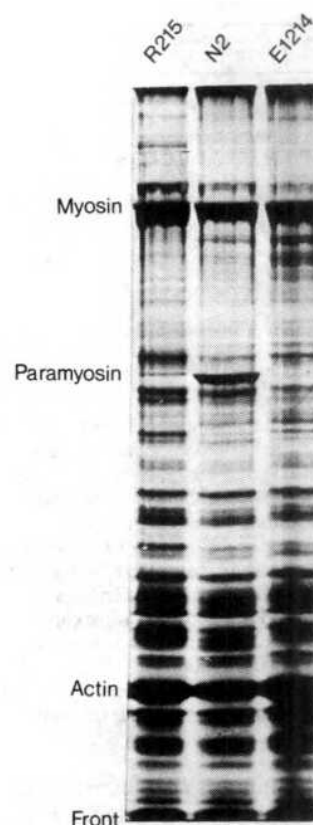


Fig. 1 Comparison of total SDS-soluble proteins from the nematode strains N2, E-R215 and E1214. Animals were grown in liquid culture, collected⁴⁰ and frozen²⁹ until use. Care was taken to obtain cultures of animals in similar larval stages to minimise possible developmental variations. 15 μ l of a 1:7 (v/v) dilution of each sample was applied to an SDS-polyacrylamide gel³⁹. The gel was stained with Coomassie brilliant blue, destained and photographed.

Fig. 2 Electron micrographs of body wall muscle from the three strains, N2, E-R215 and E1214. Adult animals grown at 20 °C were prepared for microscopy as previously described²⁹, and transverse sections were viewed and photographed with an AEI EM5B electron microscope. Magnification 30,000 $\times$.

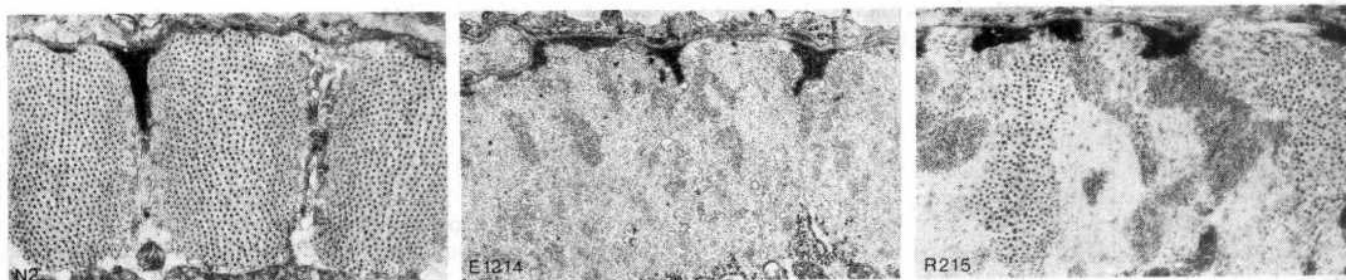


Table 2 Mutation suppression by *e1464*

Gene	Phenotype	Alleles suppressed by <i>e1464</i>	Alleles not suppressed by <i>e1464</i>
<i>unc-15 I</i>	Muscle defective (paramyosin)	<i>e1214</i>	<i>e73</i>
<i>unc-54 I</i>	Muscle defective (myosin heavy chain)	<i>e576, e1108</i> <i>e1021, e1008,</i> <i>e1419</i>	<i>e190, e569, e651,</i> <i>e675, e903,</i> <i>e1009, e1092,</i> <i>e1115, e1168, e1201,</i> <i>e1258, e1274, e1300,</i> <i>e1315, e1328, e1420,</i> <i>st1, st2, st3</i> <i>e309, e323, e328,</i> <i>e973, e1038, e985,</i> <i>e387, e51, e158</i> <i>e499</i>
<i>unc-13 I</i>	Severely uncoordinated	<i>e312, e376,</i> <i>e450, e1091</i>	<i>e903</i>
<i>dpy-18 III</i>	Dumpy	<i>e364</i>	NA
<i>cat-1 X</i>	Catecholamines lacking neuronal processes	<i>e1111</i>	NA
<i>nuc-1 X</i>	Lacks deoxyriboendonuclease	<i>e1392</i>	NA

Doubles of the suppressor with each of the above autosomal mutations were constructed by the elimination of markers closely linked either to the suppressor or to the mutant to be tested. For example, E1464 males were mated with the double mutant *unc-54; sma-2*, and six paralysed or slow F_2 progeny (those homozygous for *unc-54*) were selected. The progeny of each animal was inspected for suppressed animals. Those animals that did not segregate small progeny were assumed to be homozygous for *e1464*, as *sma-2* is less than 2% from *sup-5*. For sex-linked mutants, suppression was evaluated directly by observing the phenotype of males produced from a cross of mutant hermaphrodites with *e1464/+* males. In the case of *nuc-1*(*e1392*), 63 of 148 male progeny were suppressed and with *cat-1*(*e1111*), 60 of 129 males had suppressed phenotypes, in good agreement with the predicted ratio. All the mutations listed, as well as those described in the text, were isolated after EMS treatment³⁶ except *e903* (X-ray mutagenesis)³⁰, *e1258* (DES mutagenesis) and *e1168* and *e1201* (ICR-22 mutagenesis) (Riddle, personal communication). Some of the mutants of *unc-15* (ref. 29), *unc-54* (refs 30, 31), *unc-13* (ref. 36), *nuc-1* (ref. 38), *dpy-18* (ref. 36), and *cat-1* (ref. 37) loci have been described previously. Only those which showed clear phenotypic suppression were scored as positive. NA, no other independent mutants are available for this locus.

has been shown to contain a small internal deletion in its heavy chain³². Of the 22 null mutants tested, five show clear phenotypic suppression. The suppressed animals move even as adults, lay eggs and attain a full adult size. Nonetheless, suppressed animals move much more slowly than wild type and are clearly distinguishable from them. Neither *e675* nor *e1274*, mutations resulting in altered proteins^{30,31}, is suppressed.

Several independent mutants that are extremely uncoordinated have been isolated in *unc-13 I*. Reversion studies had been previously carried out with mutants in *unc-13* and several alleles had been found to be suppressible by unlinked suppressors (S.B., unpublished). Of 13 alleles tested, four are suppressed by *e1464*. When both the suppressible *unc-13* allele and *e1464* are homozygous, animals have nearly wild-type movement; suppression is also apparent in animals homozygous for the *unc-13* allele but heterozygous for the suppressor. The suppressor mutation is semidominant. To test the effect of temperature on the suppressed strains, all the double strains have been grown at 15 °C and 25 °C. In all cases, the suppression is more pronounced in animals grown at 15 °C, consistent with the idea that the difference in suppression at the two temperatures is associated with the suppressor *per se*. These observations correlate with the increased sterility and slow growth noted at 15 °C in the E1464 strain.

Lastly, mutant alleles of two genes that have no relationship to muscle function or structure have also been tested. One, *cat-1*(*e1111*) X, alters the distribution of formaldehyde-induced fluorescence in catecholamine-containing neurones³⁷, and the other, *nuc-1*(*e1392*) X, results in the loss of detectable deoxyriboendonuclease activity³⁸. Phenotypically, the latter mutation results in the persistence of pyknotic nuclei of cells undergoing programmed cell death and in the presence of undigested DNA in the lumen of the digestive tract; these alterations are easily detected in Feulgen-stained animals. Clear suppression of both mutations was found when the suppressor was present only in a single dose (Table 2).

Mechanism of suppression

Because the protein products of *unc-15* and *unc-54* genes are known, suppressible mutations in these genes facilitate the determination of the molecular consequences of suppression. The E1214 strain contains essentially no paramyosin and virtually no normal thick filaments in the body wall muscle cells²⁹

(Figs 1, 2). When the total SDS-soluble proteins from the suppressed strain R215 are analysed by polyacrylamide gel electrophoresis, a faint band with a mobility similar to the paramyosin band from N2 is present, whereas among the E1214 proteins no such band is seen (Fig. 1). In addition, a protein has been purified from E-R215 showing mobility identical to wild-type paramyosin on SDS gels, but the yield of paramyosin is only about 10% of that obtained from wild type. Paracrystals have been prepared from R215 paramyosin and, when viewed with the electron microscope on negatively stained grids, they seem to be similar to paracrystals of wild-type paramyosin. Cyanilation cleavage of the E-R215 protein with 2-nitro-5-cyanobenzoic acid⁴¹ results in five fragments which have the same mobility on SDS gels as those obtained from wild-type paramyosin.

The structure of E1214 body wall muscles is also affected by the suppressor. By polarised light microscopy, E-R215 shows an increased birefringence, mostly localised in poorly defined, nearly longitudinal bands (presumably A-bands). In electron micrographs of transverse sections, E-R215 displays several of the ultrastructural features of E1214, but in addition, clusters of filaments having the diameter and appearance of wild-type thick filaments are present among abundant thin filaments. These thick filaments are much less numerous and less well ordered than in N2 (Fig. 2).

Preliminary analysis of the suppressed myosin mutant strain *unc-54*(*e576*); *sup-5*(*e1464*) indicates partial restoration of wild-type muscle structure. Myosin has been purified from the strain, but because myosin heavy chains in *C. elegans* are the products of more than one gene^{30,31}, it is more difficult to determine if the *unc-54* myosin is restored by the suppressor. However, cyanilation cleavage yields peptides that are unique products of the *unc-54* heavy chain³¹, and initial experiments indicate partial restoration of these peptides in *unc-54*(*e576*); *sup-5*(*e1464*). The large number of mutants in this gene may allow us to determine whether the restoration of gene product by the suppressor always correlates with phenotypic suppression.

Conclusion

Our experiments show that *e1464* suppresses mutants of several different genes, including two that code for muscle proteins, as well as others that are clearly not related to muscle. In the genes

in which several independent mutations are available, only a few of the mutants are suppressible; most are unaffected by *e1464*. The extent of phenotypic suppression is different in mutants of different genes: *unc-15* and *unc-54* mutants are weakly suppressed, whereas with *dpy-18*, *unc-13*, *nuc-1* and *cat-1* mutants a nearly wild-type phenotype is restored. These phenotypic differences probably reflect the functions of the gene products, as the weakly suppressed mutants involve proteins that are required stoichiometrically to form the muscle lattice. One of the well-suppressed mutants, *nuc-1*, affects an enzymatic function, and perhaps the others do also.

In E-R215, the suppressor partially restores the specific gene product (paramyosin) affected by *e1214*. This is also apparently the case with the suppressible myosin mutants. It is likely that in the double mutant *sup-5(e1464); nuc-1(e1392)*, endonuclease activity has been restored, but no direct tests have been carried out.

The suppressor is semidominant, or dose dependent. The heterozygous suppressor *e1464/+* is clearly effective in the strongly suppressed mutants, and a slight effect of the heterozygous suppressor can also be detected in *unc-15* and *unc-54* mutants. It should be feasible to demonstrate some restoration of paramyosin in *e1214/e1214; e1464/+* animals. The suppressor alone exerts a deleterious effect on the animal, particularly at low temperatures, where suppression of mutant phenotypes is greatest.

None of the *unc-15* and *unc-54* mutants which contain altered gene products is suppressed and the suppressor seems to act only on a subset of null mutants. We do not yet know why the null mutants are devoid of gene product. If ethylmethane sulphate produces principally G to A transitions in the nematode as it does in microorganisms⁴², then nonsense mutations producing premature chain termination are likely to account for most of the large set of null mutants. As yet, no truncated fragments have been identified in these mutants, but it is possible that they are degraded after formation or lost in purification. The mutant E675 does contain a shortened chain, but this results from a small internal deletion which leaves the C-terminus intact³². Thus the *e1464* mutation may be a specific nonsense suppressor analogous to those found in microorganisms, perhaps acting through an altered tRNA species. On the other hand, the suppressible mutations may affect some other step of information transfer and the suppressor may alter another component of the machinery. Further genetic and biochemical studies should reveal which of these possibilities is correct.

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letters to nature

Contribution of intermediate luminosity X-ray galaxies to the background: Anon 0945-30

EVIDENCE that the galaxy A0945-30 is a new extragalactic X-ray source has been found from an analysis of rotating modulation collimator (RMC)¹ quick-look data collected from 53 orbits of the SAS 3 X-ray observatory during the period 26 February-2 March 1978. The exposure is approximately $7 \times 10^6 \text{ cm}^2 \text{ s}$. The galaxy lies within the error boxes quoted for 4U0945-30 and 2A0946-310 but is not one of the previously

suggested candidates. A faint star alongside the galaxy also lies in the 90% confidence X-ray source error circle (Fig. 1). The data are consistent with a point source². The flux from the source varied by a factor of about five during the period of our observation.

The SAS 3 RMC data alone do not allow a unique spectral fit. Fortunately, data from HEAO 1, (R. F. Mushotzky, personal communication) give a photon number index $\gamma = 1.8 \pm 0.1$ and a hydrogen column density of $N_H = 3 \pm 1 \times 10^{22} \text{ cm}^{-2}$ for a power law spectrum extending to about 35 keV. Using a 2 keV low-energy cutoff the SAS 3 data yield a slope identical with the HEAO 1 value. The X-ray flux density is $(4.5 \pm 0.6) \times 10^{-29} \text{ erg cm}^{-2} \text{ s}^{-1} \text{ Hz}^{-1}$ at 10^{18} Hz (4.14 keV) and the 2-10 keV

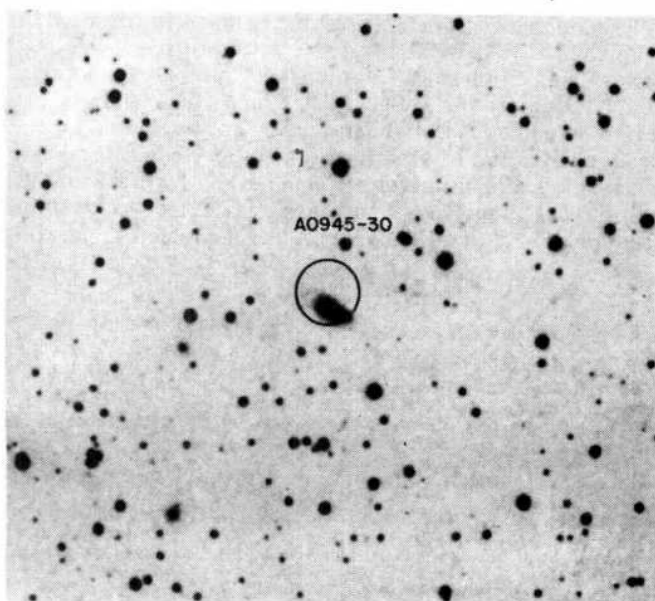


Fig. 1 The SAS 3 90% confidence error circle (radius = 35") superimposed on a photograph of A0945-30. The measured position of the X-ray source is $\alpha(1950) = 9\text{ h } 45\text{ min } 28.4\text{ s}$, $\delta(1950) = -30^\circ 42' 36''$ and the position of the galaxy is $\alpha(1950) = 9\text{ h } 45\text{ min } 28.43 \pm 0.13\text{ s}$, $\delta(1950) = -30^\circ 42' 57.0 \pm 1.7''$.

X-ray flux is $(7.7 \pm 0.3) \times 10^{-11} \text{ erg cm}^{-2} \text{ s}^{-1}$. The measured heliocentric radial velocity is $2,498 \pm 15 \text{ km s}^{-1}$ and, with $H_0 = 50 \text{ km s}^{-1} \text{ Mpc}^{-1}$, the average X-ray luminosity is $\sim 2.2 \times 10^{43} \text{ erg s}^{-1}$.

The hydrogen column density inferred from the X-ray spectral cutoff indicates that the galaxy A0945-30 rather than the star is the X-ray source. In the source direction, the hydrogen column density in the Galaxy is at most $\sim 2 \times 10^{21} \text{ cm}^{-2}$ (refs 3, 4), nearly an order of magnitude less than the column density obtained by Mushotzky (personal communication).

Recent observations by Liller (personal communication) indicate that the galaxy is an early type spiral (possible an Sa) with a bright nucleus. Detailed optical observations of the galaxy and the star were made with the new digital spectrograph on the 1.5-m telescope at Mt Hopkins observatory. The star is an F dwarf with no indication of emission features. Plates in the Harvard collection covering this field show no evidence for long term variability of the star (L. Chaisson and J. P. Huchra, personal communication). Spectra of the galaxy in the red (4,500–7,000 Å) and blue (3,900–6,000 Å) show sharp, intermediate strength emission lines of [OIII], [NII] and H α together with strong Mg, Fe, Ca absorption features characteristic of a normal stellar continuum in the galactic nucleus (Fig. 2). The absorption-corrected absolute magnitude of the galaxy is ~ -20.0 . No significant H β emission is present. The other emission lines present are just resolved at the instrument resolution of $\sim 200 \text{ km s}^{-1}$ FWHM or 300 km s^{-1} FW0I (full width zero intensity) (Fig. 2). This width is considerably smaller than the widths of 1,800 and 1,100 km s^{-1} FW0I reported by Ward *et al.*⁵ for H β in the X-ray emitting galaxies NGC2992 (2A0943-140) and NGC7582 (2A2315-428). The width is also smaller than the $\sim 1,800 \text{ km s}^{-1}$ FW0I for H β (refs 6, 7) in NGC 5506 (refs 8, 9). NGC 2992, NGC 7582 and NGC 5506 are similar to weaker type 2 Seyferts. The emission line equivalent widths are much smaller in A0945-30 than in NGC 5506 (20 Å as opposed to 200 Å for $\lambda 5007$ Å) which could be due to larger extinction of the nuclear source; the correction for dilution by the galactic continuum in our long slit (4" $\times$ 25") observation only increases the emission line equivalent widths for A0945-30 by a factor of ~ 2 . The excitation of both galaxies is quite high with $I(\lambda 5007)/I(\text{H}\beta) \sim 10$ in NGC5506 and ≥ 20 in A0945-30.

Our optical observations of the galaxy A0945-30 strengthen the case for its identification with the X-ray source. Previously, it would have been unreasonable to identify A0945-30 with 4U0945-30 or 2A0946-310 because of the large area error boxes associated with these non-modulation collimator observations. Identifications would be made only when well-known objects which are members of established classes of X-ray emitters were in the error box.

A0945-30, NGC5506, NGC2992 and NGC7582 are members of an X-ray optical luminosity class characterised by X-ray luminosities of $\sim 10^{43} \text{ erg s}^{-1}$ and with $-20.5 \leq M_B \leq -19.0$. The number density of all galaxies in this absolute magnitude range is $\sim 10^{-2} \text{ Mpc}^{-3}$ (ref. 10). The $10^{43} \text{ erg s}^{-1}$ X-ray galaxies could, therefore, be numerous and it is interesting to speculate on the possibly significant contribution of

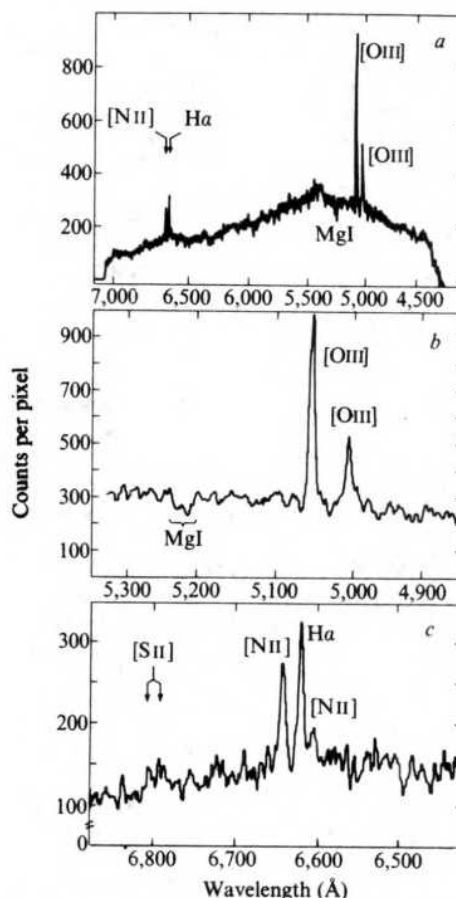


Fig. 2 *a*, A spectrum of A0945-30 obtained during a 15-min integration with the new digital spectrograph on the 1.5 m Mt Hopkins telescope. *b* and *c*, enlarged sections of the spectrum showing prominent emission and absorption features. The data have been smoothed to the resolution limit of about 5 Å. The detector has a resolution of ~ 1.8 Å per pixel.

objects in this range of the X-ray-optical luminosity function to the X-ray background.

In the 1–20 keV range the X-ray background intensity $I(E) = 8.5E^{-0.5 \pm 0.2} \text{ keV (keV}^{-1} \text{ cm}^{-2} \text{ s}^{-1} \text{ ster}^{-1})$ (ref. 11). If the emissivity of extragalactic X-ray sources per unit co-moving coordinate volume is independent of cosmological epoch, then already-known classes of discrete sources (Seyferts, quasars, clusters of galaxies) can account for at most 40% of the X-ray background¹². The X-ray luminosity density required to account for the remaining 60% of the background intensity is $\sim 10^{39} \text{ erg s}^{-1} \text{ Mpc}^{-3}$. The required number density of $10^{43} \text{ erg s}^{-1}$ sources is 10^{-4} Mpc^{-3} . To account for the background, only 1% of galaxies in the magnitude range $-20.5 \leq M_B \leq -19.0$ need be $10^{43} \text{ erg s}^{-1}$ X-ray emitters.

The background spectrum ($\alpha = 0.5 \pm 0.2$) in the 1–20 keV range could be produced by discrete sources with a range of power law spectra. One consistent model for the distribution of spectral indices is a gaussian with mean spectral index $\bar{\alpha} = 0.5$ and dispersion $\Delta\alpha = 0.4$ (ref. 13). The spectra of A0945–30 ($\alpha = 0.8 \pm 0.1$) NGC5506 ($\alpha = 0.3 \pm 0.3$) and NGC7582 ($\alpha = 0.3 \pm 0.4$) (R. Mushotzky, personal communication) are all in the expected range.

Uhuru observations reveal fractional fluctuations in the 2–10 keV background intensity of $\delta I/I = 3.6 \pm 0.5\%$ for an effective solid angle of $\Omega \approx 0.004$ ster (ref. 11). If the 10^{43} erg s⁻¹ sources are randomly distributed with mean density 10^{-4} Mpc⁻³, the expected $\delta I/I \sim 2\%$, consistent with the observational limit. If these X-ray emitting galaxies are clustered in the same way as the general galaxy population¹⁴, the fluctuations are increased by at most a factor of 2.

The X-ray luminosities of individual clusters of galaxies ($\sim 10^{44}$ erg s⁻¹) (ref. 12) provide another constraint on the acceptable fraction of galaxies like A0945–30. Photometry of four X-ray clusters^{15,16} indicates that the expected mean number of 10^{43} erg s⁻¹ X-ray galaxies is at most one per cluster.

The SAS 3 RMC survey covers $\sim 5\%$ of the sky to a limiting flux of $\sim 2.5 \times 10^{-11}$ erg s⁻¹ cm⁻². A 10^{43} erg s⁻¹ source could be detected at a distance of ~ 60 Mpc. If the mean density of sources is 10^{-4} Mpc⁻³ the survey should have detected 4–6 sources in the $1\text{--}2 \times 10^{43}$ erg s⁻¹ range. The detection of three sources (A0945–30, NGC5506, and NGC7582) indicates that these galaxies may be sufficiently numerous to account for $\sim 50\%$ of the X-ray background. The number of sources required is also consistent with the number of unidentified sources in the high latitude ($|b^{\text{U}}| > 20^\circ$) Uhuru (ref. 17 and C. Jones, personal communication) and Ariel¹⁸ surveys which have limiting 2–10 keV sensitivities of 5×10^{-11} erg cm⁻² s⁻¹ and 6.1×10^{-11} erg cm⁻² s⁻¹ respectively. We expect Uhuru detection of 20–30 of these $1\text{--}2 \times 10^{43}$ erg s⁻¹ sources and Ariel detection of 15–20 such sources. To produce $\sim 50\%$ of the X-ray background intensity we require less than half of the completely unidentified high latitude sources in either survey to lie in this intermediate X-ray–optical luminosity range.

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SS Cygni as a hard X-ray source identified with the scanning modulation collimator on HEAO 1

X-RAY emission from the dwarf nova SS Cygni was first detected at soft ($\sim \frac{1}{2}$ keV) X-ray wavelengths when the source was in an optical high state^{1–3}. More recently, ANS satellite observers⁴ have reported the detection of soft X-ray emission when SS Cygni was in an optical low state. These latter observations show the flux of the soft X-ray source to be about an order of magnitude less than the values when in the optical high state. The soft X-ray positional uncertainty is ~ 0.2 deg². The correlation between optical and soft X-ray behaviour convincingly identifies SS Cygni as a soft X-ray source. Hard (> 1 keV) X-ray emission from a $15' \times 1.5''$ area containing SS Cygni was also reported by the ANS observers during the optical low state and identified with SS Cygni⁴. A more accurate position (0.03 deg²) has been reported by Ariel 5 observers⁵. We report here a 0.4 arc min² measurement of the position of the X-ray source with the HEAO 1 modulation collimator experiment which leads to an unequivocal identification of SS Cygni as an X-ray emitter in the 2–10 keV energy range. We have also searched the Uhuru data to investigate the historical light curve in hard X rays.

The +Y axis instruments of the HEAO 1 satellite were pointed at SS Cygni for two orbits on 16 December 1977, when it was in an optical low state. The modulation collimator (MC) experiment data were binned⁶ about the position of SS Cygni. We used only one orbit of data (~ 1 h of observing time) because of problems in the determination of the aspect for the second orbit. Moreover, we rejected an additional 18% of the data when there were rapid changes in the background level.

The ANS and Ariel 5 error boxes were used to reduce the multiplicity in our positional determination. Five MC diamonds (Fig. 1) are within the ANS error box and only one, which includes SS Cygni (Fig. 2), is in the Ariel 5 error box. The area within this diamond is 0.4 arc min². This is a more than 200-fold improvement with respect to previous results^{4–5}, and definitively confirms that SS Cygni is a hard X-ray emitter.

The MC flux (2–6 keV) is $(1.7 \pm 0.5) \times 10^{-10}$ erg cm⁻² s⁻¹ with an additional 30% systematic uncertainty. The fit of the three A-3 spectral channels to a bremsstrahlung spectrum with Gaunt factor and low energy cut-off gives an equivalent temperature $kT > 10$ keV and a low energy cut-off $< 0.2 \times 10^{22}$ N_H cm⁻².

Oscillations of small amplitude (< 0.002 mag) with a period of 9.75 ± 0.03 s have been reported in the optical flux of SS Cygni during an optical maximum (Robinson, personal communication). We searched our data for periodicities, by folding according to different trial periods and calculating the χ^2 value for the hypothesis of a constant flux. We did not find any significant enhancement in χ^2 that could be attributed to the presence of pulsations for periods in the range 5–20 s. The 3σ upper limit for the pulsed fraction is $\sim 13\%$ for periods consistent with that of the optical pulsations.

We have investigated the historical light curve in hard X rays by analysing all the relevant Uhuru data. SS Cygni was in the Uhuru field of view ($0.5^\circ \times 5^\circ$ FWHM) and not confused with nearby sources in 12 d of observations. These data are scattered over 1.5 yr, but they are always coincident with an optical low state. In two instances there was marginal evidence for source detection in the 2–6 keV band: at an intensity of $(4.4 \pm 2.7) \times 10^{-11}$ erg cm⁻² s⁻¹ on 20 June 1971 and at $(9.0 \pm 3.2) \times 10^{-11}$ erg cm⁻² s⁻¹ the following day. These are of the same order as the ANS and HEAO 1 fluxes. Ariel 5 observed SS Cygni for a period of 120 d (ref. 5) including both low and high optical states. While the equivalent 2–6 keV flux during low states is highly variable between 7 and 30×10^{-11} erg cm⁻² s⁻¹, in the optical high state there is only an upper limit (2σ) of 6×10^{-11} erg cm⁻² s⁻¹.

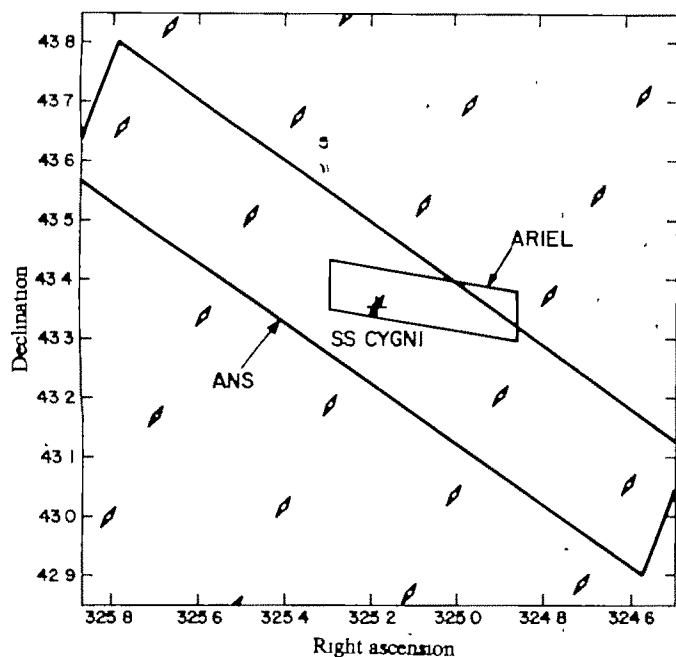
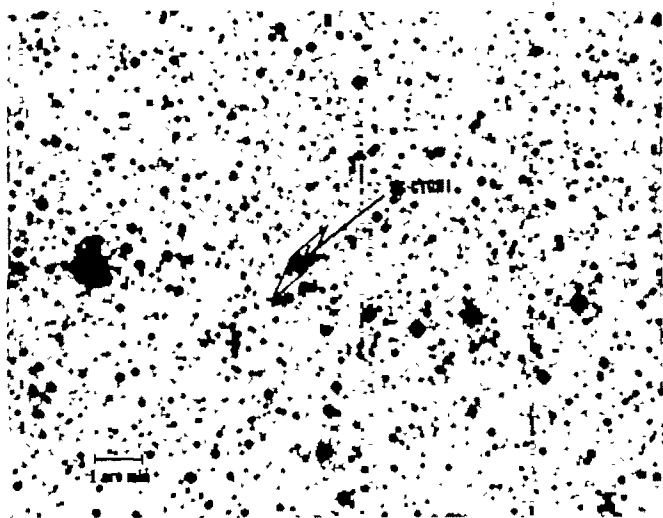


Fig. 1 One of the multiple MC positions (diamonds) falls within the previous location uncertainty measured by ANS (ref. 4) and Ariel 5 (ref. 5). This region has an area of 0.4 arc min^2 , and contains SS Cygni near its centre

The X-ray luminosity of SS Cygni in the 2–10 keV energy range is then of the order of $10^{33} \text{ erg s}^{-1}$ (for a distance of $\sim 200 \text{ pc}$) in the optical low state and probably at least an order of magnitude less in the high state. In contrast, the soft X-ray luminosity increases by an order of magnitude between the optical low and high states: from 2 to $30 \times 10^{31} \text{ erg s}^{-1}$ respectively. The average spectral behaviour (0.25–10 keV) of SS Cygni thus shows two distinct states correlated with the optical behaviour: the effective spectrum seems to soften during the optical high states.

SS Cygni is the brightest of the dwarf novae, with a visual magnitude ranging between 12.1 at minimum and 8.1 at maximum (see reviews by Glasby⁸ and Robinson⁹). Its light curve shows outbursts that repeat with an average period of 50.6 d, but with a range of periods between 33 and 71 d. It is a spectroscopic binary system composed of a normal late type star orbiting about a white dwarf with a period of 6 h 38 min. The masses of both

Fig. 2 Enlargement of the POSS region containing SS Cygni, showing the MC location precision.



components are estimated to be in the range $0.8\text{--}1.0 M_{\odot}$. The continuum emission seems to be yellow at the minimum of the light curve and blue-violet at the maximum. The spectrum is characterised by hydrogen emission lines at the beginning of the rise to the optical maximum and hydrogen absorption lines that become broader while the luminosity reaches the maximum.

It is believed (see Paczynski in Preprint No. 73 of the Polish Academy of Sciences) that the white dwarf and its accretion disk are the source of the optical and ultraviolet as well as the soft X-ray emission in the dwarf novae. There is observational evidence that links the emission and absorption lines to the accretion disk⁹. The soft X-ray flux could either originate from the boundary layer of the disk and the white dwarf¹⁰ or from the surface of the disk which could behave as a corona¹¹ (B. Paczynski, personal communication). The limited information on the hard X-ray emission does not permit us to discriminate between these two hypotheses. A coronal model can explain the inverse correlation of the soft and hard X rays which is also true if accretion on to the white dwarf is the production mechanism of the hard X rays. In the coronal hypothesis the soft and hard X-ray fluxes both originate in the accretion disk, due to the interaction of magnetic fields and plasma, analogous to the solar corona. As discussed by Galeev *et al.*¹¹ The hard X-ray flux can be produced in magnetically confined plasma (loops arising from the disk surface) while the soft X-ray flux arises from regions of less intense magnetic activity. The increase in the soft X-ray flux, which could result from an increase in the accretion rate during the optical maximum, would cause a decrease in the hard X-ray flux by comptonisation. If accretion on to the white dwarf is the production mechanism of the hard X rays, as in the model of Fabian *et al.*¹², the anticorrelation of the soft and hard fluxes could be explained by a variation in the optical thickness of the region emitting hard X rays. This might result from the increased density of the accreting gas during the optical outbursts. The soft X-ray flux, which originates further away from the surface of the white dwarf, is not likely to be affected in the same sense. Instead, the increase in the temperature of the accretion disk during optical maximum could enhance the soft flux. The accretion mechanism for SS Cygni is discussed in detail by Ricketts *et al.*⁵. The lack of a cut-off at the low energies in the hard X-ray spectrum of SS Cygni is consistent with the $\sim 35^\circ$ inclination of the orbit of the system and with accretion from a disk.

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Long-term variability of galactic X-ray sources

THE long-term (days to months) variability of 32 persistent galactic X-ray sources brighter than Cas A in the energy range 3–10 keV is discussed here, using data from the MIT experiment on the OSO 7 satellite¹. For each X-ray source, we compiled a list of counting rates for all observations made during the first 600 d of operation of the experiment² (typically six or seven observations for each source). The results in these lists show the well-known high variability of the massive early-type X-ray binaries. Of special interest, however, are the variability characteristics of the sources associated with old populations. These include several near the galactic centre which are generally referred to as 'bulge' sources, and several which are known to lie in globular clusters. They share certain similar X-ray characteristics, namely, spectra which are markedly softer than those of massive binaries and an apparent absence of pulsations and eclipses^{3–6}. Because of their similarities, it has been suggested that they are all systems of the same kind, such as neutron stars in low mass binary systems^{7,8}, or the products of core collapse in globular clusters⁹. However, our results show that the long-term variability of the bulge sources is generally much less than that of the sources which lie in globular clusters. This suggests that there is a substantial difference between the bulge and globular cluster sources either in their nature or, at least, in certain critical properties of their components such as the masses of the nuclear-burning companions which may supply the material for accretion by compact objects in binary systems.

Only those sources at galactic latitudes in the range $|b^{\text{II}}| < 10^\circ$ were included in our study. An average counting rate was derived for each observation of each source. A typical observation lasted several days and was separated from other observations of the same source by periods ranging from a few days to a few months. The duration of each observation was sufficiently long to average out the effects of eclipses of the X-ray binaries. We considered only data from our 3–10-keV detectors because they were largely uncontaminated by solar X rays or trapped particles. For this study we selected a subset of sources that were sufficiently bright so that variations of intensity >10% would be discernible. Specifically, we chose those sources for which, during at least one observation, a counting rate greater than two standard deviations above 2.5 counts s^{-1} was measured. This intensity is roughly that of the Perseus Cluster and the Cas A X-ray sources, generally believed to be nonvariable and which could, therefore, be used as standards (see ref. 2 for details).

For each source we computed a parameter which measures the variability from observation to observation. This parameter is defined by

$$\xi = \alpha / \bar{x} \quad \text{where} \quad \bar{x} = \frac{\sum_i (x_i / \sigma_i^2)}{\sum_i (1 / \sigma_i^2)}$$

$$\text{and} \quad \alpha = \left(\frac{\sum_i (x_i - \bar{x})^2 / \sigma_i^2}{\sum_i (1 / \sigma_i^2)} \right)^{1/2}$$

where x_i is the counting rate for each observation and σ_i is the

statistical uncertainty associated with each counting rate. The summations are carried out over all the observations of the sources in question. $\bar{x}$ is the mean counting rate and α is the r.m.s. deviation of the counting rates from this mean. (Each intensity measurement is weighted by its errors, see ref. 10.) ξ , defined as $\alpha / \bar{x}$, represents a measure of the fractional fluctuation of the x_i about $\bar{x}$. (We have not considered, in detail, the intensity distribution functions of all the sources.)

We also computed a parameter defined by

$$\Delta\xi = \bar{\sigma} / \bar{x} \quad \text{where} \quad \bar{\sigma} = \frac{\sum_i 1 / \sigma_i}{\sum_i 1 / \sigma_i^2}$$

$\bar{\sigma}$ is taken to be the magnitude of typical errors in the intensity measurements. The parameters of all the sources were computed for the two sets of data collected by the detectors behind the 1° and 3° collimators separately¹.

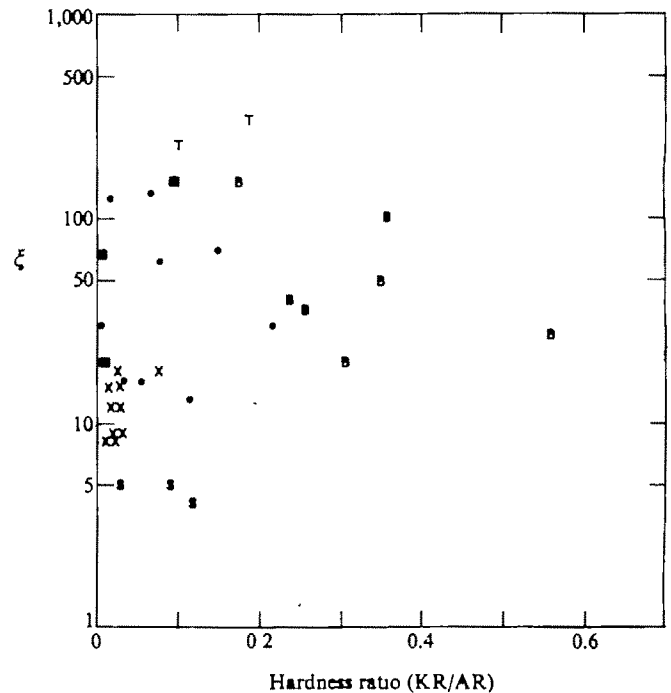


Fig. 1 The variability measure ξ is plotted against the spectral hardness (KR/AR) for the sources listed in Table 1. Standards (S) are the Crab Nebula, the Perseus Cluster and Cas A; GB (x), galactic bulge sources; GC (■), sources in globular clusters; B, binary X-ray sources; and T, transient X-ray sources. Those sources in Table 1 that are not yet known to be one of the above classes are plotted as dots.

Table 1 summarises the results on all the sources that satisfy the above selection criteria. The value of ξ listed for a source is the higher one from the two data sets. If the values of ξ measured for a source are comparable in the two sets, the one with a smaller $\Delta\xi$ is listed. Also listed are the value of $\Delta\xi$ and the number of observations (ν) for that source. We emphasise that $\Delta\xi$ should not be viewed as an error for ξ but merely as an indication of the counting statistics in the various measurements. For an X-ray source with constant intensity, the expected value of ξ is $\sim \Delta\xi$. The sources in Table 1 are ranked in increasing ξ ; also included are the Crab Nebula, the Perseus Cluster and Cas A which are generally believed not to vary significantly on

Table 1 Variability characteristic of galactic X-ray sources

X-ray source	ξ ($\times 100$)	$\Delta\xi$ ($\times 100$)	ν	Detector	Class*	Comment
3U0531+21	0	4	5	3		Crab Nebula
3U0316+41	0	5	6	3		Perseus cluster
3U2321+58	5	5	5	3		Cas A
3U1744-26	8	7	6	3	GB	GX3+1
3U1758-20	8	7	6	3	GB	GX9+1
3U1758-25	9	4	6	3	GB	GX5-1
3U1728-16	9	1	7	3	GB	GX9+9
3U1811-17	12	8	6	3	GB	GX13+1
3U1813-14	12	1	6	3	GB	GX17+2
3U1624-49	13	21	11	1		$l^{\text{II}} = 335^\circ$, $b^{\text{II}} = 0^\circ$
3U1735-44	15	9	10	1	GB	Burst source
3U1642-45	15	6	7	3	GB	GX340+0
3U1636-53	16	6	8	1		Burst source
3U1837+04	16	5	5	3		Ser X-1, burst source
3U1755-33	18	9	6	3	GB	
MX1709-40	18	8	9	1	GB	
3U1746-37	20	13	6	3	GC	NGC 6441
3U1956+35	20	10	4	1	BI	Cyg X-1
3U0900-40	27	9	4	3	BI	Vela X-1
MX1608-52	30	13	11	1		
3U2142+38	31	1	3	3	B	Cyg X-2
3U1728-24	36	7	7	1	B	GX1+4, pulsar
3U2030+40	40	7	5	3	B	Cyg X-3
3U1223-62	50	25	5	1	BI	GX301-2, pulsar
3U1820-30	63	13	6	1	GC	NGC 6624, burst source
3U1658-48	67	7	11	1		GX339-4
3U1320-61	70	30	5	3		
3U1258-61	103	14	5	3		GX304-1, pulsar
3U1705-44	127	38	7	3		Burst source (?)
3U1516-56	132	15	2	3	B	Cir X-1
MX1746-20	152	12	4	1	GC	NGC 6440
3U1118-60	153	24	4	1	BI	Cen X-3
3U1908+00	218	6	5	3		Aql X-1, transient (?)
3U1630-47	231	50	8	1		Recurrent transient
3U1543-47	304	51	12	1		Transient

* GB, galactic bulge; GC, globular cluster; B, binaries (?); BI, binaries associated with young massive primaries.

the time scale under study. The observed values of ξ and $\Delta\xi$ for these sources indicate that they are nonvariable (within an uncertainty $\Delta\xi$). As systematic errors affect the results for weak sources more than those for strong ones, we limited our study to sources brighter than Cas A.

Figure 1 shows the relationship between long-term variability and spectral hardness as characterised by the ratio of the counting rate in the 15–40-keV channel to that in the 3–10-keV channel². As expected, Table 1 and Fig. 1 show the high variability of young massive binary sources (BI). In contrast, the bulge sources (GB) are, for the most part, either steady or only slightly variable. The six least variable sources (excluding the 'standard' sources) are GB sources. The absence of long-term variability is, therefore, a distinguishing characteristic of the bulge sources.

The sources in globular clusters (GC), on the other hand, vary in about the same degree as the young binary sources. Although the sample of GC sources is small, two of the three in our list are much more variable than any of the GB sources. Three other globular cluster sources, NGC1851, NGC6712 and M15, which failed to make our list because of their low intensities, are also known to be highly variable¹¹.

Sources of type I X-ray bursts are found both inside and outside globular clusters (for example, the burst source associated with the globular cluster NGC6624 (refs 12, 13) and MXB1735-44 which is identified with an isolated faint blue star¹⁴⁻¹⁶; for detailed properties of X-ray burst sources, see ref. 17). It seems likely that the globular cluster sources and all the type I burst sources, both inside and outside globular clusters, form a class of similar objects (F.L. and G.C. in preparation).

They could, for example, be non-magnetised neutron stars in low-mass binary systems (see ref. 18 for a model). If the bulge sources near the galactic centre are, as we conjecture, of the same nature, then the systematic difference in the long-term variability must be attributed to differences in some critical parameter(s) affecting the mass transfer process. One such parameter, for example, may be the mass of the nuclear burning companion. The typical mass of the nuclear burning companion of an X-ray binary in the core of a globular cluster would be $\sim 0.5 M_\odot$. Low-mass X-ray binaries formed outside globular clusters such as by the evolution of cataclysmic variables¹⁹ may have nuclear burning companions with masses of $\sim 1.0 M_\odot$.

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Definition of a black hole

TIPLER has given a new definition for a black hole¹. These objects had previously been properly defined only in asymptotically flat spacetimes², but it seems unlikely that our Universe is one of these. One of the well known properties of a black hole, proved in asymptotically flat spacetimes, is that its 'surface area' can never decrease. By means of this new definition Tipler has shown that this area theorem may well not be true in our Universe. However, Tipler's definition is complicated, and it is not obvious that it corresponds to our usual concept of a black hole. I show here that it is equivalent to a definition that is both simpler and more clearly a formalisation of what we mean by a black hole.

I use the notation of refs 1 and 2. Let T be the set of points of a stably causal spacetime which belong to non-cosmological trapped surfaces. Tipler defines a black hole as the closure of the topologically-smallest future set I^+ such that I^+ contains T , and the boundary of I^+ is generated by (future inextendible) null geodesic segments which are boundary generators of TIPs.

We would like a black hole to contain T . It should not be possible to escape from a black hole, so the hole should also contain $I^+(T)$. Furthermore, any points from which it is impossible to avoid falling into a black hole might as well be said to be within that hole. That is, a black hole should be the closure of $B = D^-(I^+(T))$. I shall show that B satisfies the conditions set out by Tipler for I^+ .

B is a future set: suppose $x \in B$ and $y \in I^+(x)$. There is a future directed timelike curve from x to y . If it meets $I^+(T)$ then $y \in I^+(T)$, so $y \in B$. If it does not meet $I^+(T)$, it must do so when extended along any future directed non-spacelike curve α from y as $x \in D^-(I^+(T))$. Therefore, any such α meets $I^+(T)$, so $y \in B$.

The boundary of B is generated by null geodesic segments which are boundary generators of TIPs: let x be on the boundary of B . Let (x_i) be a sequence of points lying to the past of x , not in B , with x as their limit point. There is a future inextendible non-spacelike curve γ , from each x_i that does not enter $I^+(T)$. By proposition 6.2.1. of ref. 2 there is a future inextendible non-spacelike curve γ from x which is a limit curve for the γ_i .

As $\bar{B}$ is a closed future set γ can never leave it. As no γ_i can enter B , γ cannot enter the interior of B . But $I^+(\gamma) \subset B$, so γ cannot enter its own future. Therefore, it must be a null geodesic.

$\bar{I}(\gamma)$ is a TIP (see ref. 3). As γ cannot enter its own future it cannot enter its own past, $I^-(\gamma)$. Therefore, it is a boundary generator of $I^-(\gamma)$.

It remains to be proved that B is the smallest such I^+ . It has been shown that from any point on the boundary of B there is a future directed null geodesic γ that does not enter the interior of B , so cannot enter $I^+(T)$. Therefore, the boundary of B is not part of B , and B is open.

Suppose there is an I^+ that is contained in B but not equal to it. We can then find a point p which is in B but not in I^+ . Let α be a future directed timelike line from p . It must meet $I^+(T)$, and hence must enter I^+ . Let it cross the boundary of I^+ at y . There is a future directed null geodesic generator γ of the boundary of I^+ from y . It is future inextendible, and cannot enter $I^+(T)$ as otherwise it would be in the interior of I^+ . Thus, by proceeding from p along α to y then along γ , we can construct a future inextendible non-spacelike curve from p which does not meet $I^+(T)$. This contradicts $p \in B$.

Therefore B is the smallest such I^+ , and the black hole defined by Tipler is exactly $\bar{D}^-(I^+(T))$.

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Neutrinos from binary pulsars

PULSARS are believed to produce high energy particles. If high energy protons produced by the pulsar strike a target, say a binary companion, high energy neutrinos are produced in the collisions. At the same time, schemes for detecting high energy neutrinos produced in nature are currently being considered (refs 1-4 and K. Lande, personal communication). Here we point out that binary systems containing moderately young pulsars (ages $T < 10^4$ yr, that is comparable to NP0532) may emit high energy neutrinos ($10^{12} < E_\nu < 10^{14}$ eV) at detectable levels. Such pulsars are assumed to have total luminosities of the order 10^{38} erg s⁻¹. Discussion of feasible neutrino telescopes (such as the deep underwater muon and neutrino detector, DUMAND¹⁻⁴) suggests that fluxes from point sources as small as 10^2 - 10^3 eV cm⁻² s⁻¹ in high energy neutrinos may eventually be detectable. Neutrino detectors on a more modest scale, such as that of Lande (personal communication) will be operating within 1-3 yr, and have detection thresholds of 10^5 eV cm⁻² s⁻¹. Thus, a pulsar emitting 3×10^{34} erg s⁻¹ in high energy neutrinos at 3 kpc would be detectable even with Lande's detector, and the DUMAND detector may be able to detect sources that are weaker by three to five orders of magnitude.

We assume that at least some of the pulsars emit a fair fraction ($\approx 1\%$) of their energy in the form of high energy protons ($10^{13} < E_p < 10^{16}$ eV). This energy range is consistent with the upper limit⁵ of the potential near the star

$$V_{\text{max}} = 10^{16} (B/10^{12} \text{ G})(\Omega/200 \text{ s}^{-1})^2 V \quad (1)$$

where B is the magnitude of the magnetic field at the stellar surface, and Ω is the frequency of rotation. That young pulsars can produce very energetic particles is implied by the very energetic ($E_\gamma \approx 10^{12}$ eV) pulsed γ rays⁶ emitted by NP0532. The energy in the protons is converted efficiently to high energy neutrinos if either the protons traverse more than ~ 50 g of material, or they traverse a column density of 10^{28} photons cm⁻² in photons that are not too far above the pion production threshold in the rest frame of the protons. In each case, the protons are stopped by pion and kaon producing processes, and the charged mesons that are produced decay into three neutrinos and an electron. Roughly half the energy in the original proton goes into high energy neutrinos, which typically have energies of the order $10^{-2} E_p$ to $10^{-1} E_p$ (ref. 3).

There are several plausible ways in which the high energy protons might encounter enough target material to convert 1% to 50% of their energy to neutrinos.

If the companion star fills its Roche lobe, it could occupy nearly half the sky as seen from the pulsar, thus blocking a proportionate fraction of the high energy protons. Moreover, the overflowing material may completely engulf the pulsar.

Accreting matter can pile up at the magnetopause (which in the case of a pulsar would be made up of very low frequency electromagnetic Alfvén waves). It has been suggested^{7,8} that such a pile up occurs in several intermediate and rapidly rotating binary X-ray sources.

Or, the accreting matter might settle into a disk much smaller than the binary orbit, and much larger than the light cylinder. Such a disk could have a random orientation relative to the rotation axis of the pulsar, and could block a fair fraction of the protons leaving along open field lines. Moreover, as the disk may be substantially heated by such protons, it is likely to have a corona that completely engulfs the pulsar.

Besides being a target itself, matter near the pulsar can scatter and trap photons that also may serve as a target. To fix the ideas, suppose that 10^{38} erg s^{-1} of X rays are emitted by the pulsar and diffuse away from it through surrounding matter. Assume the scale height of the photons is 3×10^8 cm and that their mean free path is λ ($\lambda < 3 \times 10^8$ cm). Then the density of photons at the scale of 3×10^8 cm is $3 \times 10^{19}(\lambda/3 \times 10^8 \text{ cm})^{-1}$, enough to stop all the protons by photopion production.

Finally, we discuss stellar winds. Suppose the pulsar's companion is an OB supergiant. Motivated by standard models of binary X-ray sources⁹, we assume a mass loss rate $\dot{M} = 10^{-5} M_{\odot} \text{ yr}^{-1}$, a wind velocity near the pulsar of $V = 300 \text{ km s}^{-1}$, and a binary orbit radius of 3×10^{11} cm. Then the column density of the wind surrounding the pulsar is of the order of (depending on direction) $1\text{--}10 \text{ g cm}^{-2}$, thereby providing a significant target. Reasonable parameters for red giants, $\dot{M} = 3 \times 10^{-6} M_{\odot} \text{ yr}^{-1}$, $v = 10 \text{ km s}^{-1}$, $R = 3 \times 10^{13}$ cm, yield a comparable column density. Pulsar emission impinging on the companion could stimulate a wind regardless of its type as has been suggested¹⁰ for the case of Cyg X3.

Assuming a pulsar formation rate of one every 10 yr (ref. 11), there ought to be $\sim 10^3$ moderately young pulsars in the Galaxy at present and more if pencil beaming effects allow a selection efficiency of only a few per cent. Older pulsars, though less powerful, are more numerous, and may also be detectable in neutrinos. Direct evidence that neutron stars can form without disrupting the binary system (such as binary X-ray sources) combined with the observation that a significant fraction of all stars (at least $\sim 50\%$ (ref. 13) perhaps most¹⁴) are in binaries allows the hypothesis that a significant fraction of these young pulsars are in binaries.

One route for binary pulsar formation particularly favourable to neutrino production is that a helium star or white dwarf, accreting the Roche lobe overflow from a red giant companion, becomes massive enough to explode, leaving a pulsar. (An explosion is not necessary, the pulsar may form quietly¹².) Just after the explosion, the companion to the young pulsar is automatically in the stage where its extended envelope and overflow play a significant role as target material.

It is difficult to predict the *a priori* probability of detecting, by conventional means, any pulsars that might exist in binary systems. First, there are beaming effects. Moreover, if there is some mechanism that accelerates pulsars to $\sim 250 \text{ km s}^{-1}$ at their inception (refs 15, 16 and refs therein), only the ones that are tightly bound—to relatively massive companions—are likely to remain in their binary orbit. Hence accretion clouds become an important factor. Most binary pulsars are unlikely to appear in radio surveys as their accretion clouds and the coronas of their companions would generally have plasma frequencies in excess of the radio. (The only binary pulsar found by Hulse and Taylor had a compact companion; one might expect many more with main sequence companions.) If they are blanketed by more than 1 g cm^{-2} , their pulsation could not be detected at any frequency, and if by more than $\sim 30 \text{ g cm}^{-2}$, no non-thermal photons are likely to escape because they would first be degraded to energies below 10 keV, where they would then be absorbed by partially ionised iron and oxygen^{13,17}.

Cyg X3, generally presumed to be a pulsar in a binary system, is blanketed by $\sim 10 \text{ g cm}^{-2}$ (ref. 10), and is deduced to be a pulsar only by virtue of its γ -ray emission. Cyg X3 itself may be detectable in high energy neutrinos if it emits $\geq 10^{35}$ erg s^{-1} in high energy protons. There may also be a whole class of objects like Cyg X3, but obscured by much thicker envelopes.

Hefand and Tademaru¹⁶ noted a distinct class of pulsars with very low transverse velocity. To account for this, they suggest

that $\sim 20\%$ of all pulsars form in close binary systems, probably in a 'cataclysmic binary' sequence such as outlined here. (The pulsar is later released gently from the binary as the companion sheds mass.) High energy neutrino astronomy would provide an opportunity to test such a hypothesis. Conversely, their hypothesis suggests that there may be many interesting astrophysical phenomena to observe with neutrino telescopes. We emphasise that if high energy particles generated by the pulsar manifest themselves by bombarding the corona or envelope of the companion star, the mesons and photons they produce are directed into the star. Hence high energy neutrino astronomy may be the only way to detect high energy phenomena in such systems.

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Constraints on the corona model for Sirius B

SINCE the discovery of Sirius as a soft X-ray source¹, two models have been proposed to account for the emission—thermal emission from deep layers of an almost pure hydrogen atmosphere², and an acoustically heated corona around the white dwarf (WD) Sirius B (ref. 3). Both models seem unsatisfactory in some respects: the thermal emission model does not agree with the recently revised upper limits found from extreme UV observations⁴, and the T_{eff} of Sirius B is much too large for H-convection. In this note we re-examine the constraints on the corona model in the light of historical records suggesting a possible pseudo-red giant phase a few thousand years ago.

Sirius B does have an H-atmosphere, and with T_{eff} being $32,000 \pm 1,000 \text{ K}$ (ref. 5), the atmosphere is stable against convection. If it is accepted that the corona is due to the dissipation of the acoustic flux produced in subatmospheric convective layers, then for Sirius B to have a corona there must be an He-convective region beneath the atmospheric H-layers. Fontaine⁶ argues that in this case the acoustic flux produced would be much too low to satisfy the energy requirement to heat the corona. However, the theory of corona formation is not well developed⁷, and the computation of acoustic fluxes is critically dependent on the theory of superadiabatic convection. Hence, if convection survives in the subatmosphere of Sirius B, we cannot exclude the corona model. In fact, Lampton and Mewe⁸ recently revised the energetic requirements for white dwarf coronae and

claim that the corona around Sirius B may exist, provided that the H-layer is very thin.

The theory of white dwarf envelopes is already sufficiently good to quantify what 'very thin' means. The H mass M_H must be larger than the mass which provides Rosseland optical depth $\tau = 1$, as we see an H-atmosphere, but smaller than the mass which does not allow He-convection to survive. We constructed atmosphere and subatmosphere models for Sirius B according to the data given in refs 9 and 10. The models consist of an H-envelope down to a fixed mass point, of a μ zone, in which the chemical composition smoothly changes from H to He (ref. 11), and of an He mantle. At the T_{en} (from 30 to 33×10^3 K) and gravity (corresponding to $M = 1 M_\odot$) of Sirius B we find $2 \times 10^{16} \leq M_H < 10^{17}$ g.

It is difficult to believe that Sirius B is born exactly with this H-envelope. We might expect that it was born with a pure He atmosphere, like DB white dwarfs, and that it accreted H from the stellar wind of Sirius A during the white dwarf lifetime ($t = 3-4 \times 10^7$ yr, ref. 12). Since it is now clear^{13,14} that He white dwarfs do not accrete from interstellar matter at the rates predicted by Bondi's accretion formula¹⁵, but the geometrical accretion rate is appropriate¹⁰, one may derive the mass loss rate ($\dot{M}$) from Sirius A which accounts for the required M_H , simply as:

$$M_H = \dot{M} \pi R_s^2 / (4 \pi d^2)$$

where R_s is the Sirius B radius (5.5×10^8 cm) and d the system separation (20 AU). This relation provides $3 \times 10^{-13} \leq \dot{M} < 2 \times 10^{-12} M_\odot \text{ yr}^{-1}$, which is not an unreasonable rate for a $2 M_\odot$ star. Therefore, the corona model for Sirius B cannot be excluded on the basis of these arguments. If the white dwarf, corona-like emission were confirmed, we should know, with an uncertainty of a factor of only 10, the rate of mass loss from Sirius A.

However, we feel that the historical record of Sirius being a red star should not be dismissed as unpalatable. Most of the references to this (Greek, Roman, Egyptian) were collected at the end of last century by Schiaparelli^{16,17}, who did not believe that Sirius could have changed colour, because, in his time, the theory of stellar evolution predicted that stars evolved from white to red, and then to total darkness—'as a piece of iron cooling from white heat'. Further historical quotations—not so complete—are given by See¹⁸, who, unlike Schiaparelli, believed that the historical evidence strongly suggested that a colour change had taken place. If the historical records are to be believed, Sirius must have changed colour from red to white between the epoch of Ptolemy (AD 100) and the epoch of Al-Sûfi (AD 980), within 800 yr.

We now know with reasonable certainty that the age of Sirius as a white dwarf cannot be so low, but we also know that hot white dwarfs are not always 'normal' stars: instabilities connected to H burning in thin shells can be found, and these may last few thousand years¹⁹. Therefore, we cannot rule out the possibility that Sirius B went through a pseudo-red giant phase a few thousand years ago.

In this context, we emphasise that the sudden expansion of the outer envelope of a white dwarf is always related to H burning, which may occur only if the H envelope of the white dwarf is large ($\geq 10^{28}$ g), and the expansion for a few thousand years is never able to completely remove this envelope. In other words, if the historical record is true, then the H envelope of Sirius B is orders of magnitude larger than the envelope required for He convection to survive, and the corona model is wrong.

Hence, if it is found that Sirius B does indeed produce a corona-like emission, and if further study of historical records and archaeological evidence indicates a pseudo-red giant phase, the theory of generation of a corona by acoustic flux dissipation must be seriously questioned. The acoustic flux model will also be in doubt, if the coronal X-emission is shown to arise from Sirius A, since Sirius A, like Sirius B, cannot have subatmospheric convection.

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If the Sun has a companion . . .

HARRISON¹ has suggested on the basis of pulsar data that the Sun might have a companion. This letter discusses some of the implications if such a companion is a compact object, more precisely a neutron star (NS) or a black hole (BH). Using existing accretion results, it is argued that it is impossible to rule out either a NS or a BH as a possible candidate. However, constraints can be placed on the allowed mass range and/or radiation mechanisms. If such a compact object exists, the probability of observing the gravitational lens effect can be substantial.

The case for a low-mass star, a black or a white dwarf has been discussed by Henricks and Staller² who concluded that such an object would be conspicuous in the visible or IR sky and would probably have already been detected. The situation for a NS or a BH is not quite so clear and one needs to consider in more detail the luminosity achieved through accretion.

Because a weakly bound binary system may not survive the explosion leading to a NS or a BH, we only consider the simple case of a close encounter between our Sun and its postulated temporary companion. The probability of such an encounter or its perturbing effects on planetary motions will not be discussed here. The accretion rate $\dot{M}$ is of the order³ $\dot{M} \approx 4 \pi G^2 M^2 \rho_0 / (a_0^2 + v^2)^{3/2}$ where ρ_0 and a_0 are the density of the unperturbed ambient gas and the corresponding speed of sound, and M and v are the mass and velocity of the compact object. As $a_0 \approx 10 \text{ km s}^{-1} (T/10^4 \text{ K})^{1/2}$ and as we expect $v \gg 10 \text{ km s}^{-1}$, the accretion rate⁴ will be of the order $\dot{M} \approx 3 \times 10^{-16} (M/M_\odot)^2 (n_0/1 \text{ cm}^{-3}) (v/10 \text{ km s}^{-1})^{-3} M_\odot \text{ yr}^{-1}$, where n_0 is the particle number density of the unperturbed gas. Taking first the case of a BH and using previous results obtained for spherical accretion from a medium at $T \approx 10^4 \text{ K}$ (as a_0 is then of the order 10 km s^{-1} , this is equivalent to considering a BH moving through the interstellar medium at $v \approx 10 \text{ km s}^{-1}$), we write the total luminosity in the form⁴⁻⁶

$$L_{\text{BH}} = \Lambda_{\text{BH}} (M/M_\odot)^3 (n_0/1 \text{ cm}^{-3})^2 (v/10 \text{ km s}^{-1})^{-3}$$

where the quantity Λ_{BH} and the spectral energy distribution depend on the details of the model. (For the models considered here, $\Lambda_{\text{BH}} \approx 10^{21} - 10^{29} \text{ erg s}^{-1}$.) As for accretion on to a NS, most (if not all) of the energy is radiated away so that $L_{\text{NS}} =$

$\eta GMR^{-1} \dot{M}$, where R is the radius of the NS. Taking $\eta \approx 0.3$ and $M/R \sim 10^5 M_\odot/R_\odot$ for a $1 M_\odot$ NS, and assuming a crude mass-radius relationship of the form $MR^3 \approx \text{constant}$, the total luminosity takes the form $L_{\text{tot}} = \Lambda_{\text{NS}} (M/M_\odot)^{10/3} (\rho_0/1 \text{ cm}^{-3})^{1/3} (v/10 \text{ km s}^{-1})^{-3}$, where $\Lambda_{\text{NS}} \approx 10^{30} \text{ erg s}^{-1}$. Again, the spectrum depends on the detailed processes by which the radiation is emitted.

Taking the distance d to the companion (as determined by the required acceleration of order $10^{-6} \text{ cm s}^{-2}$ for the Solar System) to be $d = 800(M/M_\odot)^{1/2} \text{ AU}$, we can now obtain the apparent visual magnitude V and expected X-ray intensity I in c.p.s. (counts per second) in the range 2–6 keV (1 count $\approx 1.7 \times 10^{-11} \text{ erg cm}^{-2}$), provided the spectral distribution of the radiation is known. For different parameters the quantities V and I vary according to

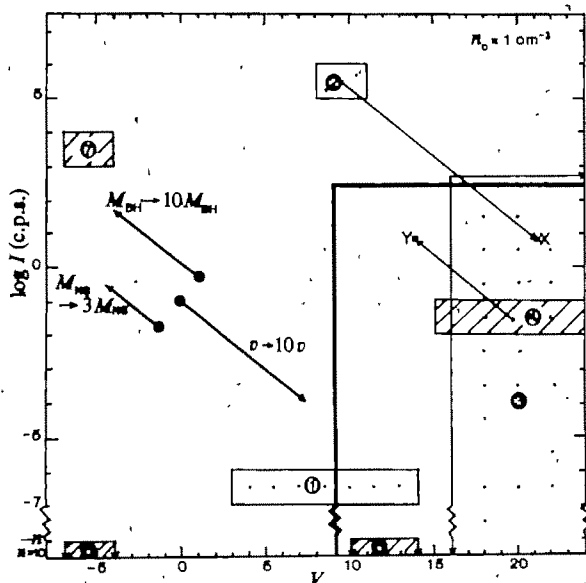
$$I = \text{const} \times M^{a-1} n_0^b v^{-3}$$

$$V = \text{const} + 2.5((1-a) \log M - b \log n_0 + 3 \log v) \quad (1)$$

with $(a, b) = (3, 2)$ for a BH and $(10/3, 1)$ for a NS. With respect to the X-ray intensity I , we note that on the basis of X-ray data⁸ there are 14 sources in the expected¹ direction of the companion, that is $17 \text{ h } 30 \text{ min} < \text{RA} < 19 \text{ h } 52 \text{ min}$, $-27^\circ < \delta < 32^\circ$. Six of these are close to the general direction to the galactic centre and thus probably associated with it, so we need not consider them. Of the eight sources left, all have an intensity $< 270 \text{ c.p.s.}$

Figure 1 shows V plotted against $\log I$ giving the results corresponding to various radiation processes around accreting NS or BH for the case $n_0 = 1 \text{ cm}^{-3}$, $v = 10 \text{ km s}^{-1}$ and $M_{\text{NS}} = 1 M_\odot$, $M_{\text{BH}} = 10 M_\odot$. The fact that each case is represented by an area instead of a point reflects the uncertainties in estimating the amount of energy emitted in the visible and X-ray bands. (The IR magnitude K also generally correlates with the V magnitude so that a bright visible object would also be a bright IR object.) The three NS results are labelled 1, 2 and 3 on Fig. 1.

Fig. 1 V magnitude and (2–6 keV) X-ray intensity I corresponding to various radiation processes, discussed in the text, around accreting NS (dotted areas 1, 2 and 3) and BH (hatched areas 4, 5, 6 and 7). The parameters used are: $n_0 = 1 \text{ cm}^{-3}$, $M_{\text{NS}} = 1 M_\odot$, $M_{\text{BH}} = 10 M_\odot$ and $v = 10 \text{ km s}^{-1}$. The three arrows on the left of the diagram illustrate the shift in the position of three arbitrary points (●) if M and v are changed as shown. Arrows leading to points marked X and Y refer to specific changes discussed in the text. An object could have possibly remained undetected until now only if its corresponding point on the diagram lies inside the region indicated by the heavy line, that is $V > 9$, $I < 270 \text{ c.p.s.}$



As the emitted spectrum is complex and depends strongly on the actual physical processes (for a review of accretion on to a NS, see ref. 7), we have not tried to represent realistic situations. Instead, we have assumed some simple radiation mechanisms to illustrate the wide range of possible results. Hence, cases 1 and 2 correspond respectively to black-body emission at $T \approx 10^5$ – 10^6 K and bremsstrahlung at $T \approx 10^6 \text{ K}$ (in the latter case, comptonisation if taken into account would augment the high-energy end of the spectrum). In case 3, a shock above the surface is assumed to heat the electrons to a high temperature of the order of 10^{12} K , and V and I are estimated by assuming that the bulk of the energy is in the form of γ rays and that the spectral distribution does not increase at lower energies. The four BH results, labelled 4, 5, 6 and 7, were obtained from earlier work on accretion. For the chosen models, the emission is predominantly due, in case 4 to radiative recombination and bremsstrahlung⁴, in cases 5 and 6 to electron synchrotron radiation^{5,6,14}, and in case 7 to bremsstrahlung, thermal and cosmic ray electron synchrotron radiation¹⁵.

Clearly, for either a NS or a BH, the results are very sensitive to the details of the emission process. In particular, a NS companion can certainly not be ruled out, especially because increasing the velocity to 100 km s^{-1} , and taking a lower mass (all observed NS masses⁹ seem to lie in the range $0.3 \leq M/M_\odot \leq 1.2$) substantially favour the possible existence of a NS companion (all points get shifted diagonally downwards parallel to the arrows on the diagram). As for a BH candidate, if Shvartzman's model or Mészáros' is the correct one (areas 6 or 7 on Fig. 1, large synchrotron luminosity including contribution from cosmic ray electrons in Mészáros' case), then a BH companion, in order not to be one of the brightest objects in the visible sky, would have to have either $v \gg 10 \text{ km s}^{-1}$ or $M \ll 10 M_\odot$ or both (for example, a $1 M_\odot$ BH moving with velocity $v = 100 \text{ km s}^{-1}$ would have $V \approx 9$). Note that a BH of $1 M_\odot$ would have to be primordial in origin. It is interesting that, taking the maximum allowed cosmological density¹⁰ of BH and a velocity of the order 100 km s^{-1} , an encounter at the required distance every 10^8 – 10^9 yr is expected independent of the mass of the BH (both the number of BH, and the square of the distance depend linearly on the mass of the BH). On the other hand, taking Shapiro's model or equivalently assuming the magnetic field to be relatively unimportant (areas 4 and 5 on Fig. 1), then one sees that a BH with $M \leq 10 M_\odot$ and $v \geq 10 \text{ km s}^{-1}$ could easily have remained undetected until now. Wright¹¹ has suggested a supermassive black hole of $M > 6 \times 10^3 M_\odot$ moving with $v > 9 \text{ km s}^{-1}$ as a possible solar companion. Compared to a $10 M_\odot$ BH, such a BH would have a luminosity larger by a factor of order 4×10^5 (corresponding to $\sim 14 \text{ mag}$). Regardless of the accretion model adopted, this BH would be very conspicuous in both or either of the visible and X-ray sky and is thus highly unlikely as a candidate.

Could one of the already observed X-ray sources be this postulated companion? Of the eight aforementioned X-ray sources listed in the 3U catalogue⁸, one, 3U1832–23, lies on the ecliptic (thus minimally disturbing the orbit of the planets). For this source $I = 6.9 \pm 0.9 \text{ c.p.s.}$ If this source were the solar companion, then we could certainly rule out cases 1, 5, 6 and 7. On the other hand, assuming the kind of simple spectra used in Fig. 1, a NS having $M \approx 1 M_\odot$, $v \approx 10 \text{ km s}^{-1}$ could possibly be a candidate but only if electrons were heated to a very high temperature (case 3, but then it should also be a strong γ -ray emitter). Another possibility would be a NS with a basically thermal bremsstrahlung spectrum at $T \approx 10^6 \text{ K}$ (case 2) provided $(M/M_\odot)^{7/3} (v/10 \text{ km s}^{-1})^{-3} \approx 2 \times 10^{-5}$ (this change of parameters would shift the position of a point in dotted area 2 diagonally to a point X at $V \approx 21$), a typical example being $M \approx 0.3 M_\odot$, $v \approx 140 \text{ km s}^{-1}$. An accreting BH with a simple bremsstrahlung spectrum (case 4) could also be a candidate if $(M/M_\odot)^2 (v/10 \text{ km s}^{-1})^{-3} \approx 200$. This resulting increase in luminosity (shift to a point Y on Fig. 1) could be achieved, for example, with a $150 M_\odot$ BH moving at $v = 10 \text{ km s}^{-1}$. Such a BH would be at $d \approx 9,000 \text{ AU}$ and would have a proper motion

$\mu_0 \approx 45 \alpha \text{ arc s yr}^{-1}$ and parallax $\pi \approx 20 \text{ arc s}$, with α the ratio of tangential to total velocity.

It has been assumed so far that a value of $n_0 = 1 \text{ cm}^{-3}$ is typical of the interstellar medium. The actual density outside the Solar System¹⁶ may well, however, be only of the order 0.1 cm^{-3} . If this is the case, the luminosities derived from accretion are then reduced by a factor of 100 for a BH and 10 for a NS, thus making any such compact object even less conspicuous.

Another aspect of interest is the question of whether gravitational lensing by the postulated companion can have observable consequences. Wright¹¹ has briefly commented on this point. We calculate here the probability that the compact object will act as a moving lens causing an angular displacement and brightening up of distant background stars. To appreciate the angles involved, a typical angle is the angular radius of the annular image of a distant source when there is perfect alignment. This angle γ_0 (half the angle α_0 used by Refsdal¹²) is given by

$$\gamma_0 = 1.47 \text{ arc s } (M/M_\odot)^{1/4}$$

where the distance-mass relation is used for the postulated companion and the fact that this distance is considerably smaller than the star-deflector distance. For a seeing disk of 1 arc s, the above relationship shows that the deflector and primary image (the only one we consider here) are separately resolved. The amplification factor, defined as the ratio of the lensed flux to the unlensed flux is given by¹² $A = \frac{1}{4}(2 + (\alpha/\beta) + (\beta/\alpha))$, where $\alpha^2 = \alpha_0^2 + \beta^2$, $\alpha_0 = 2\gamma_0$, and β is the angular separation between the position of the deflector and that of the source in the absence of light deflection ($\beta = 0$ for perfect alignment). The corresponding magnitude change is $\Delta m = 2.5 \log A$. The angular position of the primary image with respect to the deflector is given by $\alpha_* = \frac{1}{2}(\alpha + \beta)$ so that the position of the image is apparently shifted by the angle $\delta = \alpha_* - \beta = (\alpha - \beta)/2$. Defining a characteristic transit time T as the time during which the magnitude of the image m_* satisfies $m - \Delta m/2 \leq m_* \leq m + \Delta m/2$, m being the unlensed magnitude, we find $T = 2(\beta_{1/2}^2 - \beta^2)^{1/2} \mu^{-1} \text{ yr}$ where $\beta_{1/2}$ is the value of β corresponding to a change $\Delta m/2$ and μ is the net angular velocity of the deflecting mass, including proper motion μ_0 and parallax π (in arc s yr⁻¹ if β is in arc s).

Given a plate limit m_{lim} , the probability P of observing an angular displacement of magnitude $\geq \delta$ or equivalently a change in magnitude $\geq \Delta m$ can be computed by summing the separate contributions P_m corresponding to background stars of magnitude m , $P_m = 2\beta\mu A_m(60)^{-4} \text{ yr}^{-1}$, where A_m is the number of stars¹³ per square degree in the visual magnitude range $m \pm \frac{1}{2}$ and β and μ are expressed in arc s and arc s yr⁻¹, respectively (the summation $P = \sum P_m$ formally extends to ∞ ; however, for stars with $m > m_{\text{lim}}$, the required change in magnitude is not Δm but $\Delta m + (m - m_{\text{lim}})$, thus giving an observable change of Δm).

Keeping all other parameters constant (such as the magnitude and direction of the tangential velocity), the various quantities scale with the mass as follows: $\pi = c_1 M^{-1/2}$, $\mu_0 = c_2 M^{-1/2}$ and hence $\mu = c_3 M^{-1/2}$, $P = c_4 M^{-1/4}$ and $T = c_5 M^{3/4}$, where the c 's are all constant. The quantity $N = PT$ where $\bar{T}$ is an average transit time (stars with $m > m_{\text{lim}}$ have a shorter transit time with a lower probability of occurrence) is entirely independent of μ and scales as $M^{1/2}$. It is a measure of the number of days in a year where gravitational lensing is in progress. (We can interpret a value of N larger than 365 as meaning that a number of order $N/365$ lensings are occurring simultaneously.)

The results of the calculations are shown in Table 1 and Fig. 2 for a $1 M_\odot$ object. The chosen net angular velocity $\mu = 550 \text{ arc s yr}^{-1}$ has the same value as the proper motion of a $1 M_\odot$ object having a tangential velocity of 10 km s^{-1} . (Its annual parallax would be 250 arc s .) These results are easily generalised to different values of the parameters by using the appropriate scaling laws.

Table 1 shows that the angular shift is a much more noticeable effect than the accompanying brightening up. For a $1 M_\odot$ object, it is also clear from Fig. 1 that, unless $m_{\text{lim}} \geq 21$, one does not expect numerous lensings giving a deflection of more than 1 arc s

Table 1 Typical parameters characterising the gravitational lens effect of a nearby $1 M_\odot$ object having net angular velocity $\mu = 550 \text{ arc s yr}^{-1}$

Δm	0.0002	0.001	0.01	0.05	0.1	0.5	1.0	2.0
α_* (arc s)	12.6	8.44	4.75	3.19	2.70	1.89	1.67	1.53
β (arc s)	12.4	8.18	4.30	2.51	1.90	0.74	0.37	1.27
δ (arc s)	0.17	0.26	0.46	0.68	0.80	1.15	1.29	1.41
T (d)	10.40	6.96	3.90	2.56	2.12	1.20	0.82	0.46

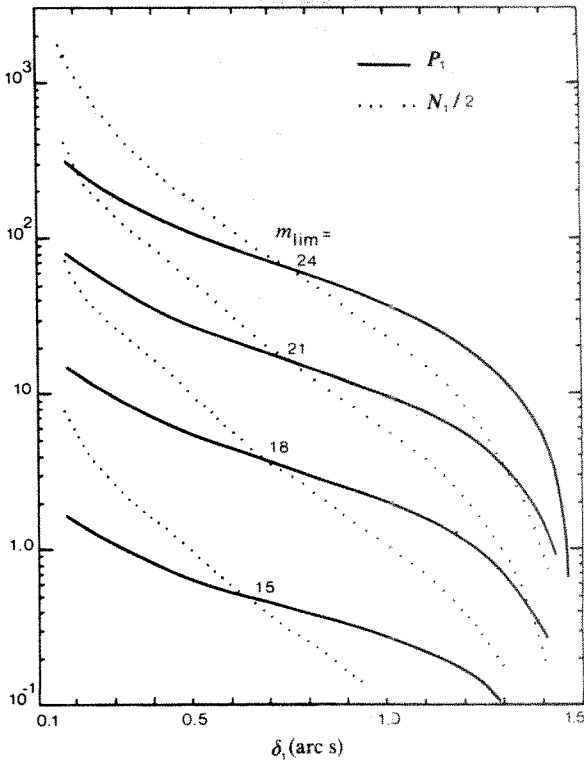
See text for scaling laws.

or $\Delta m \approx 0.1$. However, the probability of observing a deflection of order 0.4 arc s (not an unreasonable value) rises to 0.8, 7, 35 and 140 per yr for limiting plate magnitudes of respectively 15, 18, 21 and 24, each transit lasting about 4 d. Hence, a $1 M_\odot$ object would be detected by (say) taking four or more plates down to $m_{\text{lim}} = 21$ at intervals of three nights.

As for a $150 M_\odot$ BH (hypothetical 3U1832-23 case), a deflection $\delta \geq 0.5 \text{ arc s}$ (corresponding to $\delta_1 \approx 0.14 \text{ arc s}$ on Fig. 2) should be observable roughly 0.6, 6, 30 and 120 times per yr for $m_{\text{lim}} = 15, 18, 21$ and 24, respectively, the transit time now being of order 515 d (about 1.4 yr). Note that the probability (transit time) increases (decreases) by a factor of 10 if we allow a tangential velocity of the order 100 km s^{-1} . On the other hand, a NS or primordial BH of $0.3 M_\odot$ will produce a deflection of the order 0.4 arc s a number of 0.8, 8, 35 and 135 times per yr with a transit time of only 1.4 d.

It should be pointed out here that all calculations are based on mean (with respect to galactic latitude) values for A_m , thus underestimating its value on the galactic plane and hence the probabilities by a factor of the order 3-4. And, of course, if the presumed compact object is brighter than the limiting magnitude, its proper motion alone will make it conspicuous.

Fig. 2 The quantity $N_1/2$ and probability P_1 of observing a gravitational deflection greater than or of the order δ_1 for a $1 M_\odot$ object (hence the subscript) having net angular velocity $\mu = 550 \text{ arc s yr}^{-1}$. The corresponding quantities for other masses, other parameters being the same are given by $P = P_1(M/M_\odot)^{-1/4}$, $N = N_1(M/M_\odot)^{1/2}$ and $\delta = \delta_1(M/M_\odot)^{1/4}$ (strictly speaking P_1 is the expected number of lensings per year).



In conclusion, a temporary companion to our Sun in the form of a NS or a BH could have remained undetected until now. Constraints can be placed, however, on the allowed type of spectrum and/or mass and velocity ranges accessible. In particular, if $n_0 = 1 \text{ cm}^{-3}$ and if the magnetic field plays an important part, we can almost certainly rule out any BH which has $M > 1 M_\odot$ and $v < 100 \text{ km s}^{-1}$. On the other hand, if an encounter with a compact NS or BH is in progress, it could be possible to observe the gravitational lens effect, especially if one knew more precisely in which direction to look. Perhaps some photographic observations of the error boxes associated with the eight aforementioned Uhuru sources would be worthwhile, starting with 3U1832-23. At worst the data obtained could be useful for alternative work such as variable star or proper motion survey; at best, one will have the satisfaction of having observed the (most likely) closest compact object.

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Evidence for long-period global oscillations of the Sun

THE results of our observations of the solar potassium resonance absorption line at $7,699 \text{ \AA}$ using integrated solar light from the McMath telescope at Kitt Peak together with an atomic beam resonance scattering apparatus are reported here. In two long runs on successive days we observed wavelength oscillations of large amplitude with a period close to 160 min. If, as we believe, these oscillations are produced by motions of the photosphere, they provide evidence for oscillations of the entire Sun. We describe our experimental method, the analysis of the observations, and the results we obtain, and compare these with previous results.

Resonance scattering of light from a beam of free atoms is an ideal technique for making precise absolute measurements of the shift in wavelength of the light relative to the reference wavelength of the beam atoms. We have used this technique previously to measure the solar gravitational redshift¹ and to observe the 5-min radial oscillation of small regions of the photosphere². We give here only a brief outline of the method as modified for our present observations.

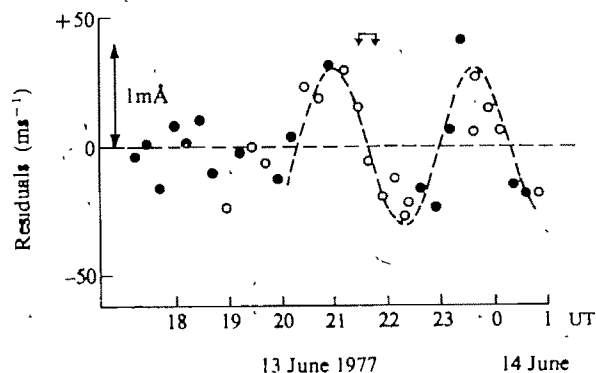
Flat mirrors placed in the main beam of the McMath telescope directed integrated sunlight vertically downwards into our apparatus, where it passed through a $3/16''$ entrance aperture, a $7,699\text{-\AA}$ interference filter with a $16\text{-\AA}$ FWHM and a motor-driven circular polariser unit which alternated the sense of circular polarisation at a frequency of about 23 Hz. The light then passed through a lens and the upper pole piece of a variable-field permanent magnet into a vacuum chamber placed

between the magnet poles where it was focused on a horizontal beam of potassium atoms produced in a nearby oven. Unscattered light was absorbed in a blackened tube placed below the beam while light resonantly scattered in a direction perpendicular to the beam passed through a short horizontal fibre optics light pipe to a dry-ice-cooled photomultiplier tube. The magnetic field was set at its maximum value, roughly 1,800 G, to produce a Zeeman splitting of the beam atom resonance absorption line into two lines whose wavelengths corresponded to the steeply sloping sides of the integrated solar potassium line. The two opposite senses of circular polarisation of the input light produced resonance scattering by beam atoms at these two different wavelengths, so that the system switched back and forth between opposite sides of the solar line at a frequency of 23 Hz. For small wavelength shifts of the solar line, the amplitude of the photomultiplier output signal at 23 Hz was proportional to the shift. This signal was fed to a Keithley 417 picoammeter whose output went to a Keithley 822 phase-sensitive detector (PSD) provided with a 23-Hz reference signal from the circular polariser unit. The d.c. output of the PSD was measured by a Keithley 172 digital multimeter and recorded by a Keithley 750 printer.

We incorporated several features designed to eliminate possible slow variations in the telescope alignment and instrumental effects. First, we diverted light from around the entrance aperture to an image-forming system so arranged that when the image was kept accurately fixed in position by photoelectric guiders the direction of the integrated light entering the aperture was also kept fixed. Second, we continuously monitored on a chart recorder the average value of the picoammeter output signal corresponding to the average of the scattered light intensity from both sides of the solar line as well as the stray light scattered from the walls of the vacuum chamber. At intervals we recorded this signal with the potassium beam turned off, to measure the stray light alone. Third, at intervals we recorded the PSD output with the beam turned off, to measure instrumental effects such as polariser asymmetry.

Our observations were made on 13 and 14 June 1977; we report here only those made during the same continuous time interval on both days. The PSD time constant was 10 s and in our data analysis we used recorded PSD outputs at 1:85-min intervals. After subtracting from each PSD output value the value with atomic beam off and then normalising it using the net beam-on minus beam-off average picoammeter output, we carried out a least-squares fit of the resulting data to the known variation of wavelength with time produced by the Earth's motion. This known variation was used to calibrate our apparatus. Finally, we averaged the residuals in groups of eight, corresponding to a time interval of 14.8 min, and plotted them in Figs 1 and 2.

Fig. 1 Residual wavelength variations of the solar potassium absorption line at $7,699 \text{ \AA}$ using integrated light. Each point is the average of eight residuals over a 14.8-min period. The dashed sine curve has a period of 160 min and its first peak occurs at 20 58 UT. The expected location of a peak, based on the data of Kotov *et al.* lies in the range indicated by the two arrows. A positive residual corresponds to ascending motion on the Sun.



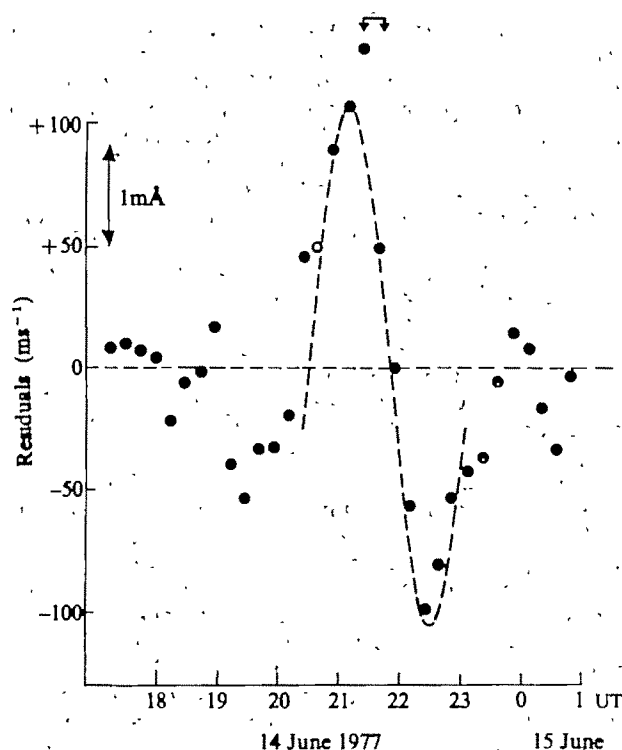


Fig. 2 The dashed sine curve has its peak at 21.10 UT. See legend to Fig. 1 for further details.

At the start of both runs the data seem to scatter randomly. Regular variations then appear with considerably greater amplitude than the noise. Kotov *et al.*³ have presented evidence for periodic variations in the wavelength of an iron line at 5,124 Å, with several different periods, one of which is 160.004 min and has an associated amplitude of about 1 m s^{-1} . These variations remained in phase over a three-year period from 1974 to 1976 and were consistent with the more limited observations of Brookes *et al.*⁴ on the potassium line with a technique similar to ours. Figures 1 and 2 show 160-min sine curves fitted by eye to our data: 160 min is apparently very closely the proper period, but the locations of the peaks only roughly agree with the expected locations based on our extrapolation of the data of Kotov *et al.* which lie in the ranges indicated on the figures by double arrows. (To make this extrapolation we assumed that a peak occurred between 4 h 47.5 min and 5 h 7.5 min UT on 4 August 1974, and that the oscillation period was 160.004 min. See Fig. 6 of ref. 3. If the period is actually 160 min then nine complete oscillations will occupy exactly 24 h so that on successive days the peaks should occur at the same times. We observed a peak on 14 June at 21.10 UT, which is 12 min later than the peak at 20.58 UT on 13 June. A possible explanation is that the period of oscillation of the potassium line is 161.3 min. This would link our data on the two successive days very nicely as well as fitting the data even better.

The power spectra calculated for these two runs showed enhanced power in the range of long periods and also other peaks but we do not trust the results because of the limited amount of data available. It seems that when there are large oscillations, obviously present in a data string, such an analysis is not very useful and is difficult to justify, particularly when each run is only three oscillation periods long. Furthermore, we are wary of attaching too great a significance to the specific periods associated with peaks in the power spectrum as the periods for which power is calculated are determined by the total data string length and are widely separated.

We believe that our method of data analysis has eliminated possible instrumental variations as the cause of the observed wavelength oscillations. We do not see how variations in the Earth's atmosphere could cause such an effect and we conclude

that we have observed a solar effect associated with global oscillation. The large amplitude of the oscillation relative to that observed by Kotov *et al.* as well as the apparent difference in phase still require explanation. Detailed discussion of the expected oscillation periods according to various solar models can be found in ref. 3 and other references listed there.

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Measurement of ^{10}Be in 1,000- and 5,000-year-old Antarctic ice

MEASUREMENT of radionuclides by nuclear accelerator spectrometry may lead to wider application of measurement of long-lived cosmogenic isotopes to archaeological, geophysical and astrophysical problems (see ref. 1 for further details of the technique). We previously² discussed the prospects for the isotope ^{10}Be and reported the successful measurement of this isotope using both the internal² and the external³ ion sources of the Grenoble cyclotron. ^{10}Be (half life = 1.5×10^6 yr; ref. 4) is formed by the interaction of cosmic rays with the Earth's atmosphere, and then precipitated out into various geophysical reservoirs such as marine sediments and polar ice⁵. The ^{10}Be profile in these reservoirs can thus be used to search for evidence of historical variations in parameters (geomagnetic field, solar modulation, primary cosmic ray intensity) which influence the deposition rate. We report here the use of the accelerator technique for the determination of ^{10}Be concentrations in samples of Antarctic ice believed to be about 1,000 and 5,000 yr old. The only published measurement for ^{10}Be in polar deposits is that of McCorkell *et al.*⁶ who made their measurement on a sample extracted from 1.2×10^6 l of Greenland ice. The present measurements were made on samples from 10 l. We believe this difference is indicative of the tremendous potential that is offered by the accelerator technique.

The samples studied were taken by a group from the Laboratoire de Glaciologie de Grenoble during the recent successful recovery of a 900-m ice core at Dome C in East Antarctica⁷. During the coring operation, a certain amount of meltwater occurs. This water was recovered and passed through cation exchange resin in the form of resin impregnated filters (Acropor SA-6404, Gelman). The resins were changed at regular intervals. Two of the cation disks were used for the present investigation. The depth and quantity of water to which they were exposed are given in Table 1.

Be was extracted from the resins by soaking them in 3M HCl for several hours (a test with ^7Be confirmed that 100% extraction was obtained in these conditions). A standardised carrier solution, containing ~4 mg of ^9Be was then added to the HCl. The mixture was neutralised and the precipitate of $\text{Be}(\text{OH})_2$ converted to BeO by heating. Pellets of sintered BeO were then fabricated by the procedure described in ref. 2 for introduction into the cyclotron ion source.

The experimental procedure was basically that described in ref. 3 using the external ion source of the cyclotron. Several of the improvements discussed in ref. 3 have been implemented,

Table 1 ^{10}Be determinations and sample details

Sample	Depth (m)	Estimated age* (yr BP)	Vol. of water (l)	$^{10}\text{Be}/^9\text{Be}^\dagger$ ($\times 10^{-13}$)	^{10}Be ($\times 10^4$ atoms per g ice)
RD-14	70.9–75.7	1,000	11.2	7.6 ± 2.5	2.00 ± 0.67
RD-18	235–242	5,000	10.2	11.2 ± 2.8	3.22 ± 0.81
Standard‡	—	—	—	8.1 ± 1.9	—

* Ages are preliminary estimates based on model calculations (C. Lorius, personal communication).

† These ratios are normalised to known standard having $^{10}\text{Be}/^9\text{Be} = 8.24 \times 10^{-12}$.

‡ This standard has a known ratio of 8.30×10^{-13} .

including replacement of the single Si detector by a $\Delta E(8\mu\text{m})$ -E telescope, a 'live-time' clock to take into account high voltage breakdowns, and rapid switching between ^9Be and ^{10}Be at the source by means of changing the extraction voltage rather than the magnetic field. In addition all beam lines have been tuned initially using $^{10}\text{B}^{2+}$ rather than $^{20}\text{Ne}^{4+}$ and this has resulted in an improved transmission efficiency. The most important change was use of a significantly more intense ($\sim 3\mu\text{A}$) output from the ion source. A more detailed description of these modifications will be given elsewhere. For the present discussion the most important effect was that we were able to improve our sensitivity by about two orders of magnitude compared to ref. 3 and so measure samples with $^{10}\text{Be}/^9\text{Be}$ ratios as small as 10^{-12} .

The analysis of the ice samples was made by comparing their $^{10}\text{Be}/^9\text{Be}$ ratio to those of standardised $^{10}\text{Be}/^9\text{Be}$ samples prepared by the method outlined in ref. 2. To avoid contamination problems measurements were made on a 'clean' ion source in the following order; standard ($^{10}\text{Be}/^9\text{Be} = 8.30 \times 10^{-13}$), ice samples, standard ($^{10}\text{Be}/^9\text{Be} = 8.24 \times 10^{-12}$). Each measurement included 20 min of ^{10}Be counting, with the ^9Be current being recorded every 5 min. A blank run gave no counts in 10 min which is equivalent to a background of less than 10% of the ice samples.

Knowing the $^{10}\text{Be}/^9\text{Be}$ ratio and the quantity of ^9Be added initially, we obtain directly the number of ^{10}Be atoms in the original sample. It is interesting to note that the chemical yield of our processing treatment does not enter into such a calculation since we are at all times working with isotope ratios. Also, as our standards have been determined by a mass spectrometric measurement², even the uncertainty associated with the ^{10}Be half-life does not intervene. On the other hand, one assumption we have been forced to make is that all the ^{10}Be initially in the meltwater has been recovered in the resin. Because of the well known tendency of carrier-free Be to hydrolyse in neutral solution and adsorb on container surfaces, this assumption needs to be verified. We hope to be able to do this in the future by measuring the ^{10}Be taken directly from an equivalent quantity of ice in the same core.

The results of our measurements are summarised in Table 1. As the ^{10}Be count rates for the ice samples were 1 to 2 c.p.m., the uncertainties are dominated by statistics. We point out, however, that this limitation in the present circumstances was due to the lack of accelerator time and not to running out of sample. In fact by weighing our samples before and after we were able to estimate that they were consumed at the rate of $\sim 0.05\text{ mg min}^{-1}$.

The present experiment is a prelude to a more general project for measuring complete ^{10}Be 'profiles' in polar ice and marine sediment cores, in order to investigate possible ^{10}Be variations with time and their eventual implications. In fact, the two ice samples in Table 1 do have a concentration difference (which is, however, not statistically very significant). It is tempting to attribute this to the difference in geomagnetic field at the two epochs. It is generally believed that the intensity of this field 5,000 yr ago was only about half of that 1,000 yr ago⁸, and thus ^{10}Be production would be expected to be enhanced at the earlier period. Indeed, this was the reason for choosing the samples

from these periods. However, in view of the limited precision of the present measurements and the fact that we are dealing with only two samples, we content ourselves for the moment with simply taking the mean of the two results as representing a long-term average for the ^{10}Be deposition rate at the site tested.

Using an estimated annual precipitation rate of 3.7 cm of water at the site of our samples (R. Delmas, personal communication), our average ^{10}Be concentration (2.6×10^4 atoms per g ice) represents a ^{10}Be deposition rate of 3.1×10^{-3} atoms $\text{cm}^{-2} \text{s}^{-1}$. This is much less than the value $2.0(+0.9 \text{ or } -0.5) \times 10^{-2}$ atoms $\text{cm}^{-2} \text{s}^{-1}$ (recalculated using the currently accepted half life⁴) measured at the Greenland site⁶. It is also 4 to 8 times smaller than the deposition rates found in most sea sediments^{9–11}. There are two possible explanations for a low ^{10}Be deposition rate at the site of our samples. First, based on measurements from bomb tests in the stratosphere, it is expected that deposition of long-lived cosmogenic isotopes at high latitudes should be substantially less than the world average⁵. In this respect, it is perhaps significant that our rate is consistent with the upper limits for the only sediment results at high latitudes¹². (Although, depending on the ^{10}Be ocean residence time, one might or might not expect such variations to be largely washed out in marine sediments due to ocean mixing.) Such an explanation would, of course, leave the Greenland⁶ result difficult to understand. Second, it is also expected that, at a given latitude, fallout rates will be roughly proportional to precipitation⁵. It will clearly be interesting to make measurements on ice samples from the coastal regions of Antarctica, where the precipitation rate is about an order of magnitude greater than at the site of the present samples, in order to determine which of these effects is dominant.

The primary objective of this study was to test whether ^{10}Be concentrations of the required sensitivity could be measured in the available ice cores (the $\sim 10^4$ atoms of ^{10}Be in each sample is several orders of magnitude smaller than could be measured by traditional beta counting, as discussed in ref. 2). In this respect the experiment was a complete success. We are thus optimistic about the chances of being able to make a complete ^{10}Be profile over the entire length of the core from which the present samples were taken (estimated to go back to 35,000 BP). If the ^{10}Be production should be as sensitive to geomagnetic field as hinted at in the present results, such a profile would offer an unparalleled opportunity for tracing the intensity variations of this field over that period. It would also allow one to look for effects such as the Laschamps–Lake Mungo–Malefif 'events'^{8,13}, which may or may not be correlated with each other and/or with worldwide dipole variations.

The present ^{10}Be determinations in samples that were unmeasured (and unmeasurable) by any other existing technique, moves accelerator spectrometry out of the phase of exploration and into that of exploitation.

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Creep rupture at depth in a cold ice sheet

THE mechanism by which stress is released in intermediate and deep focus earthquakes and the physics of tectonic processes are of particular interest to Earth scientists. As a way of understanding such phenomena it would be valuable to have a relatively small scale tectonic system duplicating the processes taking place within the Earth. Several authors^{1–3} have speculated that processes operating within glaciers and ice sheets are analogous to those in the Earth. Experimental evidence has not supported the hypothesis of an Earth glacier equivalence, especially in terms of identifying faulting at depth within glaciers. A seismic survey made of a valley glacier in Canada⁴ found no deep ice events. However, evidence of the existence of discrete shear planes within the Antarctic Ice Sheet⁵ and evidence described here relating to the Greenland Ice Sheet, indicate that faulting takes place at depth in cold ice sheets.

At great depths beneath the surface of the Earth (500–700 km) where the pressure is of the order of 10^{10} Pa and high homologous temperatures prevail, classical laws of brittle failure do not apply. As an alternative to stress release by brittle fracture, rupture of the material may result when creep is concentrated in very narrow bands subjected to shear stresses. Consideration of the creep rupture phenomenon and its associated stress drop have led to some current models of fault mechanisms for earthquakes⁶.

The mechanism by which stress is released in intermediate and deep focus earthquakes is of particular interest to seismologists and tectonophysicists. Orowan¹ proposed that faulting at depth is initiated by the development of creep instabilities in shear layers in the Earth. The stress drop theory associated with Orowan's faulting mechanism serves as a partial base for models describing the seismic spectra from earthquakes.⁶ To verify his theory, Orowan¹ proposed that this faulting mechanism might also operate in temperate glaciers.

As a means of verifying the creep-rupture theory, Neave and Savage⁴ carried out a seismic investigation on the Athabasca Glacier in Canada. They aimed to detect shear faulting at depth within the glacier. After 390 h of seismic monitoring they concluded that creep instabilities which would be recorded as deep focus ice-quakes had not taken place in the Athabasca Glacier.

Field investigation of glaciers are generally conducted where simple flow regimes occur, so that research findings can be generalised^{7–9}. However, recent drilling at the margin of the Greenland Ice Sheet^{10,11} in a peculiar area characterised by upslope flow of cold ice over geometrically complicated bedrock (see Fig. 1), has revealed the presence of a fracture at 240 m depth in the ice. The evidence for the fracture is threefold. First, although drilling was temporarily stopped at 250 m depth surface meltwater was observed to enter the liquid-filled hole, thus showing that the drilling fluid was running out of the hole at some depth. As no crevasses, moulins or englacial conduits are present in this area, this behaviour can only indicate the existence of an open fracture that intersects the hole between the surface and 250 m depth. The hole was then completed to bedrock at 299 m. Second, following drilling the saltwater used as a drill fluid was spontaneously flushed from the upper 240 m of the hole by surface meltwater whereas the lower 59 m of the hole retained the salty water, as shown by temperature measurements made periodically after the completion of drilling¹⁰. Third, calculated temperature profiles¹² for four other

holes in the same area are in excellent agreement with measured temperatures, but the calculated profile for the hole in question agrees equally well with the measured temperatures only if the ice is assumed to flow over an area of 'dead ice' as shown in the Fig. 1. Direct observations of dead ice have been made in valley glaciers but not in cold ice sheets.

As no evidence of a pre-existing fracture was obtained, it is possible that the excess hydrostatic pressure of the drilling fluid ($\sim 2.4 \times 10^5$ Pa) and the flow shear stress caused the formation of the fracture along a zone of weakness between the dead and moving ice. Whether or not there was a pre-existing fault at this depth, the excess hydrostatic pressure caused propagation and opening of the fracture sufficient to accept a large quantity of water; hence, it is likely that the fracture propagated to bedrock around the area of dead ice (the subglacial tills are permeable in this area¹⁰). The small excess hydrostatic pressure could not have caused fracture without an existing zone of creep-enhanced weakening at this depth.

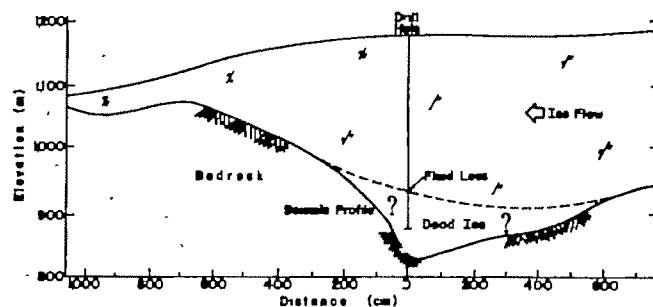


Fig. 1 Ice thickness and bedrock profile (from a seismic survey) is shown along the flow line through the drill hole, Isua, Greenland.

The geometry shown in Fig. 1 suggests an area of dead ice overridden by the ice sheet while temperature and water flow observations clearly show a fracture in the ice at 59 m above the bed. This evidence for fracture at depth in the Greenland Ice Sheet leads us to reconsider the correspondence between flow and rupture at depth in the Earth and in cold ice sheets as suggested earlier^{1,2}. If seismic evidence of deep fracturing in cold ice sheets is found we will have a large-scale tectonic system available for the study of deep fractures associated with the underthrusting of cold lithospheric plates¹³. Direct investigations at depth in ice sheets are made with relative ease as compared to the nearly impossible task of direct measurements in the Earth's mantle.

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Loss of fixed nitrogen from soils by nitric oxide exhalation

LABORATORY studies¹⁻⁸ have indicated that chemical denitrification processes in nitrogen rich soils and nitrite solutions can produce NO or NO₂ and perhaps methyl nitrite⁹. It has been suggested¹⁻⁶ that self decomposition of nitrite produces various gaseous nitrogen compounds including nitric oxide (NO) and it was thought that NO was unlikely to escape from the soil because in aerobic soils NO is readily oxidised by molecular oxygen to NO₂ and this in turn is rapidly adsorbed by soil materials and by soil water^{1,2}. However the half life of oxidation is dependent on the NO concentration because it is a termolecular reaction^{10,11}. At concentrations of 100 p.p.m. or greater (as in some studies) the half life for oxidation of NO to NO₂ by atmospheric oxygen is one hour or less respectively, whereas at low concentrations (~0.01 p.p.m.) the half life for this oxidation is of the order of 10⁴ h. This variation in the oxidation rate by molecular oxygen explains why NO at low concentrations in the field can pass unoxidised from the soil to the atmosphere and perhaps explains why in some of the laboratory studies (at high concentrations) NO₂ and not NO is detected. This NO exhalation in the field could not be detected previously due to the lack of a sufficiently sensitive measurement technique. We report here the first measurements indicating that NO is continuously exhaled from soils including ungrazed, unfertilised grassland. The measured rates of exhalation from the soil to the atmosphere are 0.06 to 0.73 × 10⁻¹¹ kg nitrogen m⁻² s⁻¹ (equivalent to 0.2 to 2.3 kg N ha⁻¹ yr⁻¹). We show that this exhalation of NO is a significant source of nitrogen oxides (NO_x) in the lower atmosphere as has been previously suggested^{12,13}. These nitrogen oxides exert a direct influence on the ambient ozone and hydroxyl radical concentrations¹⁴ which in turn pre-

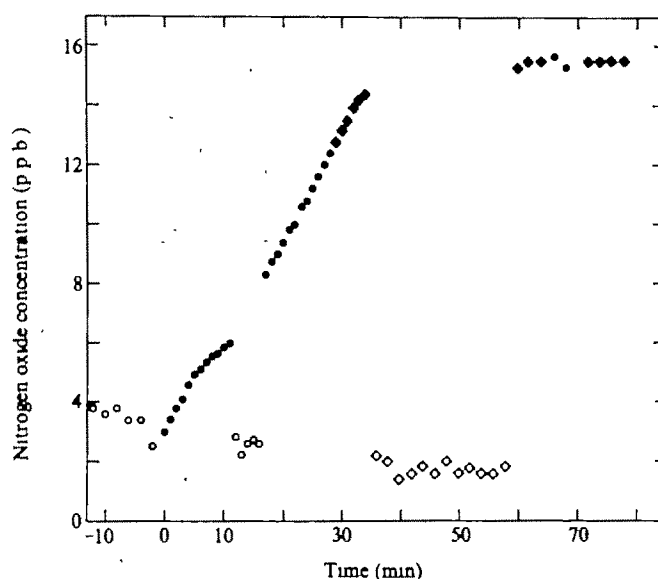


Fig. 1 Exhalation of nitrogen oxides at Aspendale on 26.11.76. The time zero corresponds to the moment of placing the box over the soil surface. ○, ambient NO_x; ◇, ambient NO; ●, box NO_x; ◆, box NO.

ester film (Dupont) to prevent gas uptake on the box walls and stirred by a 0.2 m diameter stainless steel fan driven by an external electric motor. The method will be described in detail elsewhere. NO and NO_x measurements were made with a sensitive chemiluminescent NO detector¹⁶. The NO_x measurement, (obtained by diverting the sample flow through a commercial molybdenum converter) gives the total gaseous concentration of NO, NO₂, alkyl nitrites and nitrates and peroxyacetylnitrate. These are accepted methods for measuring low concentrations of nitrogen oxides in ambient air^{18,19}.

Measurements were made at three locations described briefly in Table 1 and in more detail elsewhere²⁰⁻²². The range of conditions covered included before and after rain, day and night and spring and autumn. On every occasion measurable exhalation was found. In all experiments there was no significant difference between the concentration of NO and NO_x in the box, indicating that NO is the dominant species exhaled from the soils studied.

Kim²⁴ measured NO₂ liberation from forest soils in Korea with a closed system consisting of a sealed plastic hood containing 0.01 M sodium hydroxide absorbent. The sodium hydroxide solution used by Kim also collects NO rather inefficiently²⁵ and his results, therefore, possibly need reconsideration. Also the presence of this absorbent can inhibit autotrophic nitrification of ammonium by removing carbon dioxide²⁶.

A typical set of results is shown in Fig. 1. Immediately after placing the box over the surface there was a monotonic increase in the NO in the box above ambient levels. Generally, the concentration in the box gradually approached a maximum or equilibrium value. This behaviour indicates that there is some competing uptake mechanism or back-reaction that, in conjunction with the exhalation process, regulates the concen-

Table 1 Description of experimental sites

Location	Land usage	Soil description ²³	Nitrogen fertiliser usage
Aspendale (38 °S)	Meteorological observation area with mown grass	Siliceous sand with minimal development pH 6.2-7.1	None
Edithvale (38 °S)	Dairy cattle grazing area	Peaty soil pH 6.7 (one measurement)	None
Conargo (35 °S)	Site 1: disused cattle milking yard	Hard setting loam soil with	1 None
	Site 2: irrigated pasture	red clay subsoil	2 None in previous 4 years
	Site 3: sheep grazing —no pasture improvement	pH 6.5-7.6	3 None

dominantly determine the oxidation rate of almost all substances in the troposphere.

We determined the NO and NO_x flux from various soil surfaces by placing an open-ended box over the soil and measuring the rate of increase in concentration of these gases in the box during the following few minutes. It is assumed that the presence of the box during this time did not impede or exaggerate the gaseous flux from the soil. The continuing increase of NO in the box after sampling is discontinued indicates that there is no artificial pressure-induced exhalation of soil air into the box. The box, similar to that described elsewhere¹⁵, was a perspex cube (0.8 m per side), internally lined with Mylar poly-

Table 2 Soil analyses at Conargo sites

Site	Soil moisture (%)	Soil pH	NO ₃ -N p.p.m.	NH ₄ -N p.p.m.
1	18.6	7.6	2.1	4.0
2	10.7	6.5	1.3	3.8
3	12.2	7.2	13.1	2.4

tration of NO in the box bringing it into equilibrium with the soil/soil-atmosphere system. Concentrations of NO greater than the equilibrium value were artificially injected into the box on some occasions. The NO concentration then always decreased back to the same equilibrium value for the site.

The soil analyses presented in Table 2 (made at the time of the exhalation experiments) show that the mechanism for NO production in soil operates at a soil pH near 7 and at the p.p.m. nitrate-N and ammonium-N levels. Analyses performed recently show similar concentrations at the Aspendale site; these analyses also showed less than 0.2 p.p.m. nitrite-N. Nitrite analyses were not performed at the time of the experiments. Our results confirm laboratory experiments^{3-5,8} where detection of volatilisation products as either NO or NO₂ occurred for neutral and slightly alkaline as well as for acidic soils.

Our experimental data and the calculated emission rates are shown in Table 3. If the average flux of $0.3 \times 10^{-11} \text{ kg N m}^{-2} \text{ s}^{-1}$ is integrated over a year then a figure of $1 \text{ kg N ha}^{-1} \text{ yr}^{-1}$ is obtained.

The fluxes observed for ungrazed and grazed pastures are 0.16 and $0.35 \times 10^{-11} \text{ kg m}^{-2} \text{ s}^{-1}$ respectively. If these are representative of globally averaged land emission rates then a

Table 3 Experimental details and results

Location and date	Local time	NO flux ($10^{-11} \text{ kg (N) m}^{-2} \text{ s}^{-1}$)	NO ₂ * flux (% of NO flux)	Rainfall in previous 24 h(mm)	Air temp. (°C)
Edithvale					
16.11.76	1140	0.22	<3%	5.0	16
17.11.76	1100	0.59	<5%	3.2	14
18.11.76	1130	0.35	<3%	3.4	14
Aspendale					
22.11.76	1700	0.06	—	0	15
23.11.76	Daytime	0.15	<3%	0	16
26.11.76	1130	0.26	<2%	0.4	19
Conargo					
Site 1					
16.5.77	1600	0.37	<2%	0	16
17.5.77	0800	0.15	<3%	0	13
Site 2					
17.5.77	1530	0.25	<3%	0	~16
Site 3					
19.5.77	0730	0.25	<8%†	7	14
19.5.77	2130	0.26	<2%	0	15
20.5.77	1030	0.73	<2%	0	~16

* Includes also alkyl nitrates, nitrites and PAN.

† The uncertainty in this measurement is large because of the presence of NO₂ in the chamber (from ambient air) at the start of the experiment.

global annual value of the order of $10^{10} \text{ kg N yr}^{-1}$ is obtained which can then be compared to the global nitrogen fixation rate of 2 to $3 \times 10^{11} \text{ kg N yr}^{-1}$ (ref. 27). The loss of NO from the soil is not a permanent loss of fixed nitrogen as this nitrogen is most probably returned to the soil as nitrates in rainwater. Thus NO exhalation may be a significant process in redistributing fixed nitrogen throughout the global biosphere.

In the Northern Hemisphere troposphere these annual emissions may be $0.8 \times 10^{10} \text{ kg N}$ which, with the anthropogenic NO_x sources of $1.5 \times 10^{10} \text{ kg N}$, are sufficient to account for the nitrate deposition in rainwater of $2 \times 10^{10} \text{ kg N}$ (ref. 13).

The emissions reported here are, per unit area, about 40 times larger than the depositions of NO₃ nitrogen reported for Antarctic ice by Parker *et al.*^{28,29}. We suggest the NO₃ nitrogen in Antarctic ice may result from the long range transport of fixed nitrogen from the continental regions of the Southern Hemisphere.

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Coaxial pure shear in Jamaican blueschists and deformation associated with subduction

A RECENT report of deformation features in high pressure-low temperature blueschists on the Ile de Groix, France, has demonstrated that the style of deformation is one of progressive, non-coaxial simple shear¹. I present here evidence that the blueschists in Jamaica underwent a coaxial pure shear deformation contemporaneous with metamorphism and suggest that simple shear deformation in blueschists is no more compatible with subduction than any other deformation style. I suggest that domains of relatively homogeneous deformation occupying tens of km³ may develop in subduction complexes.

Metamorphic rocks in Jamaica consist of a narrow, NW-SE trending belt of metagreywackes and metabasites metamorphosed in blueschist, greenschist and amphibolite facies conditions^{2,3}. The belt is situated along the southern edge of the Blue Mountain massif in eastern Jamaica and the blueschists lie at the eastern end of the belt about 30 km ENE of the city of Kingston (for exact location see ref. 3). The metamorphism was the result of subduction which probably occurred in the late Cretaceous^{2,3}, but the only isotopic ages available are from biotite and hornblende in the amphibolite facies rocks⁴. The blue amphibole crossite occurs in the eastern extremity of the belt and is associated with pumpellyite, stilpnomelane, albite, clinozoisite and sphene. All CaCO₃ identified so far is calcite. Lawsonite does not seem to occur, either because the pressure/temperature gradient was not sufficiently high or because of high CO₂ pressure during metamorphism⁵. Some rocks are not entirely recrystallised and contain relict igneous augite.

The blueschists typically show an L-S tectonic fabric which plunges approximately 30° to the NNW. This orientation is not

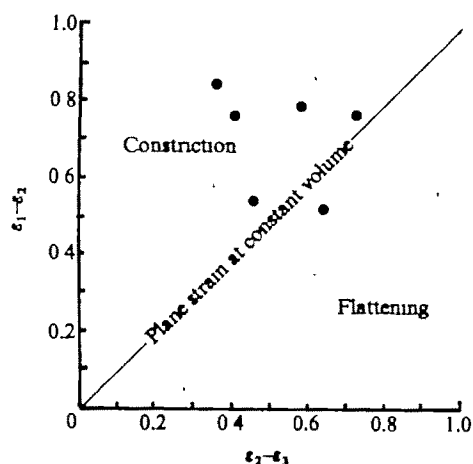


Fig. 1 Logarithmic strain plot of finite strains (ϵ) from L-S tectonites of Jamaican blueschist terrain. Most fall in the field of constriction.

consistent because the rocks have undergone further deformation associated with Tertiary E-W left lateral motion on the northern Caribbean plate boundary which lies to the north of Jamaica. The lineation itself is due to stretching parallel to the x-axis of the finite strain ellipsoid. Ellipsoidal, microcrystalline sphene blebs are probably derived from alteration of original ilmenite. A statistical study of these in individual specimens^{6,7} provides finite strain estimates, most of which fall in the field of constriction of Fig. 1.

Evidence for the coaxial nature of the strain history is exhibited by the relict augites which have undergone paracrystalline microboudinage. Amphibole fibres which have grown epitaxially between boudins are zoned continuously from riebeckitic actinolite to crossite (Fig. 2). The x-axis of the finite strain ellipsoid, amphibole fibres and elongation as defined by the boudinage are all parallel. These textural features are not unique to Jamaica and have been reported from Washington, US^{8,9}.

With continuing strain either the material properties of the rock changed or there was a change in the rate of strain, as further strain was taken up heterogeneously by the formation of discoidal ductile 'fractures' or tears. The planes of the tears are perpendicular or subperpendicular to x and are filled with tremolite and quartz fibres parallel to x. Tears are up to 0.1 m in thickness and 0.4 m in diameter and seem to be distributed randomly through the rock at intervals from a few centimetres to

Fig. 2 Sketch from thin section cut parallel to x-z plane of finite strain ellipsoid, showing typical microboudinage texture developed in metabasites. px, relict augite; g, glaucophane/crossite; a, riebeckitic actinolite; p, pumpellyite; s, ellipsoidal sphene blebs. Scale bar, 1 mm.



tens of centimetres. They have contributed to a further 5–10% extension in the x direction. This pattern of deformation is found throughout the entire blueschist terrain (about 12 km²).

The progressive coaxial pure shear reported here contrasts with the progressive simple shear reported from the Ile de Groix¹. Both these modes of deformation were contemporaneous with high pressure–low temperature metamorphism which most workers believe is produced in the crustal portions of subduction zones^{10,11}. On the megascopic scale the regional pattern of deformation is one of simple shear as the lower plate moves under the upper. However, on a finer scale, deformation is likely to be more heterogeneous. Regimes of simple shear may well exist in the rocks overlying the downgoing plate, but there is no evidence to suggest that they persist throughout the whole accretionary prism. Consideration of the irregularities in the topography of the downgoing oceanic plate and variations in the geometry of the subduction zone along strike suggest that both the simple shear of the Ile de Groix rocks and the pure shear of the Jamaican rocks are special cases and that most syn-metamorphic deformation in blueschist terrains is likely to be heterogeneous at least on a regional scale. The relatively homogeneous but different styles of deformation developed in the Jamaican and Ile de Groix terrains are present in a volume of rock of tens of km³. Do deformation domains on this scale develop in subduction complexes, or is heterogeneity developed on a finer scale in other blueschist terrains? Mechanisms of blueschist emplacement ('obduction') will be equally varied and complex. Thus, many patterns of strain in blueschists may be compatible with subduction and emplacement. More comparative studies are needed before general statements can be made.

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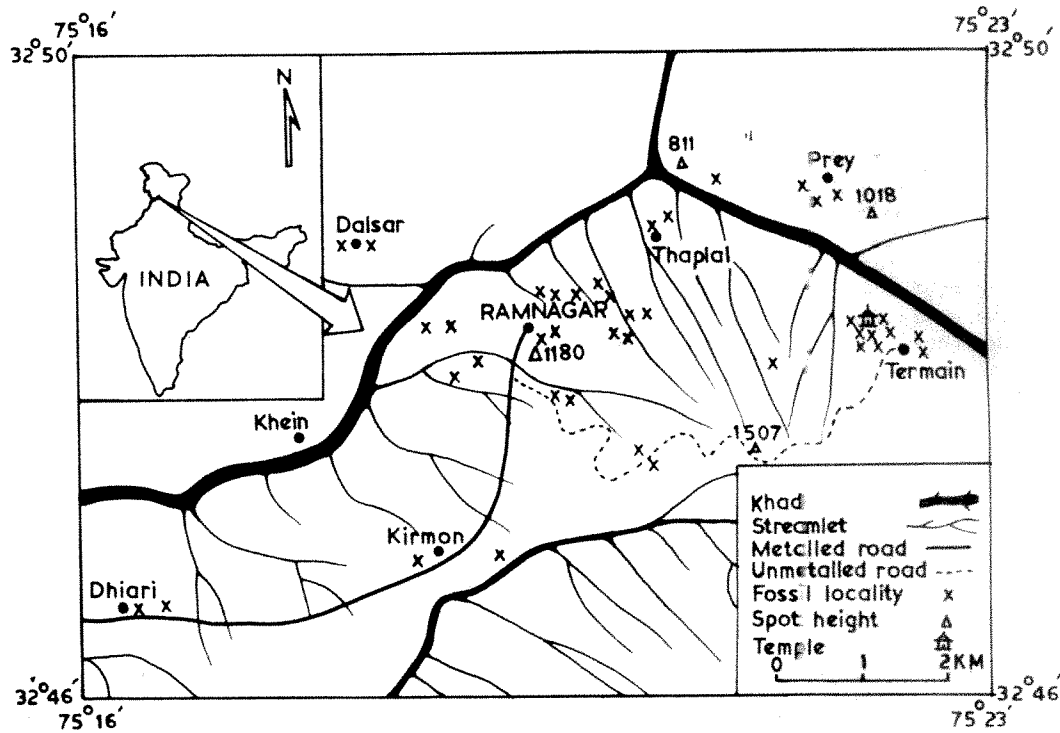
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Geology, fauna and palaeoenvironments of Lower Sivalik deposits around Ramnagar, India

ALTHOUGH the Sivalik deposits of the Ramnagar basin (Jammu and Kashmir, India) have been known to palaeontologists and palaeoanthropologists for about 100 yr, little has been reported about the fauna, flora and palaeoenvironments of the region. Most reports centre round the primates^{1–2}, and no attempt has been made to report the other vertebrates in detail. We surveyed the Ramnagar area during the field seasons of 1975–77, and catalogued more than 600 fossil specimens representing a diversity of mammals, reptiles and fish, recovered from several localities shown in Fig. 1. We report those finds here.

The Ramnagar sediments contain fauna characteristic of the Chinji Formation of the Lower Sivaliks. These deposits can be correlated biostratigraphically with the Vindobonian of Europe and possibly the Fort Ternan deposits of East Africa³ which have been assigned a potassium-argon date of 12–14 Myr. No radiometric dates are available for the Chinji deposits at Ramnagar but they can be assigned an age equivalent to 11–13 Myr on the basis of their fauna.

Fig. 1 Generalised local-ity map of Ramnagar.



The outcrops at Ramnagar show a remarkable uniformity in lithology. The beds, which are best exposed along streamlets and road cuttings, consist of alternate layers of argillaceous and arenaceous sediments, the latter dominating the former. The

Table 1 Fossil vertebrates from Ramnagar

Pisces	
Siluridae	indet.*
Reptilia	
Chelonia	<i>Trionyx</i> sp.
Crocodilia	<i>Lissemys</i> sp.
	<i>Crocodylus</i> sp.
	<i>Gharialis</i> sp.
Mammalia	
Primates	
Hominidae	<i>Ramapithecus punjabicus</i>
Rodentia	
Rhizomyidae	<i>Rhizomyoides sivalensis</i> *
	<i>Rhizomyoides punjabiensis</i> *
	<i>Sayimys sivalensis</i> *
	indet.
Ctenodactylidae	
Muridae	
Carnivora	
Creodonta	<i>Dissopsalis</i> sp.*
Fissipeda	<i>Vishnufelis</i> sp.*
Amphicyonidae	<i>Amphicyon</i> sp.*
Lutrinae	indet.
Proboscidea	
Gomphotheriidae	<i>Gomphotherium</i> sp.*
Deinotheriidae	<i>Deinotherium pentapotamiae</i> *
Perissodactyla	
Rhinocerotidae	<i>Caementodon</i> sp.*
	<i>Chilotherium? intermedium</i> *
	<i>Brachypotherium</i> sp.*
	<i>Aceratherium perimense</i> *
Artiodactyla	
Suidae	<i>Listriodon pentapotamiae</i>
	<i>Conohyus chinjiensis</i>
Anthracotheriidae	<i>Anthracotherium punjabiense</i> *
Tragulidae	<i>Dorcabune anthracotheroides</i> *
	<i>Dorcabune hyaemoschoides</i> *
	<i>Dorcatherium majus</i>
	<i>Dorcatherium minus</i>
Giraffidae	<i>Giraffokeryx punjabiensis</i>
Bovidae	<i>Protragocerus</i> sp.*
	<i>Miotragocerus</i> sp.*
	<i>Gazella</i> sp.*

* This is the first report from Ramnagar.

clayey units are greenish to dark pinkish and show evidence of nodular weathering. The sandstones contain a high percentage of undecomposed rock fragments and are frequently cross-bedded. The upper surface of sandstones is marked with asymmetrical ripples and a network of desiccation fissures in many places.

During the survey numerous fossilised fragments of various mammals were collected (Table 1). Detailed descriptions of the fossils are not available and the identifications should be considered provisional, although in broad outline they are likely to stand. In addition to primates a diversity of proboscideans, perissodactyls and artiodactyls were found. No specimen of *Hipparion* was collected and among the rodents, apart from a few rhizomyoids, only a single tooth of a ctenodactylid and a partial skull of a murid were recovered.

The general nature of the sediments is indicative of deposition in a flood-plain. The presence of red and brownish coloration in many rock units suggests that a considerable portion of sediments was deposited in oxidising and possibly warm and humid conditions. Intermittent desiccation is indicated by the occurrence of desiccation fissures on the surface of arenaceous units. The common occurrence of undecomposed rock fragments in sandstones implies short or rapid transportation, which in turn implies deposition of the sediments close to the source area.

The mammalian fossils consist mainly of suids, small artiodactyls and primates. Their abundance provides indirect evidence for the existence of general forested conditions in the vicinity of the depositional basin. The deinotheres and primitive gomphotherids are thought to have lived on soft vegetation and may have inhabited areas adjacent to water bodies and abounding in succulent and fleshy plants. The abundance of tragulids like *Dorcatherium* and *Dorcabune* implies the presence of bushy forest undergrowth near the site of deposition.

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Natural source of tetraalkyllead in air

THE biological methylation of inorganic mercury in sediments in both aerobic and anaerobic conditions is well known¹, and attention has focussed on the possibility of a similar process for lead in the environment. It has been possible to methylate lead in laboratory studies, with the resultant evolution of gaseous tetramethyllead²⁻⁴, but there is still considerable doubt as to whether such processes occur in the environment⁵. A report⁶ that tetraalkyllead accounts for 10-24% of the total lead in the liver of fresh and frozen samples of cod and 39% of the total lead in mackerel muscle lends credence to the possibility. However, we can now present firm evidence of a natural source contributing tetraalkyllead to the atmosphere.

Our evidence was obtained from a monitoring programme carried out between August 1977 and January 1978, during which we determined 24-h average concentrations of particulate lead and gaseous tetraalkyllead in duplicated air samples collected on 24 separate days, and 48-h averages obtained on four occasions, at three remote rural sites around Lancaster selected because of their freedom from local vehicular pollution sources. Particulate lead was collected on a 0.45-µm membrane filter (Millipore type HAWP 04700) and determined by flameless atomic absorption spectrometry after extraction into acid solution⁷. Tetraalkyllead was trapped quantitatively downstream of the filter in iodine monochloride solution and the lead concentration was determined by flameless atomic absorption spectrometry after selective extraction into acid solution, by means of a technique specific for tetraalkyllead compounds^{8,9}.

To interpret our results (Table 1) it is necessary to consider the known sources of particulate lead and tetraalkyllead in rural air. Furthermore, while the data can be examined in terms of their absolute concentration, it is of more value to consider the concentrations of tetraalkyllead as a percentage of total lead, as these figures depend less on site characteristics and meteorological conditions and facilitate comparison between data¹⁰. Particulate lead and tetraalkyllead in the atmosphere are due principally to emissions from vehicles fuelled by leaded petrol. These emissions arise predominantly in urban areas. Harrison and Perry¹⁰ have reviewed available data on tetraalkyllead concentrations in these urban source areas (Table 1). High concentrations of tetraalkyllead can develop around petrol stations and in underground car parks, where there is considerable evaporative loss of petrol. However, tetraalkyllead as measured by reliable techniques is more typically present at lower concentrations in urban air, representing only a small proportion of

total lead, usually about 1-4%. We made further measurements of tetraalkyllead in air, finding it to represent 4.5-5.7% of total lead close to a busy street in Lancaster city centre, when an activated carbon trap method was used,¹¹ and 0.22-0.46% of total lead alongside a remote section of the M6 motorway in Cumbria, measured as 24-h averages by the sampling and analytical method⁹. Thus we conclude from the available data that in the absence of a strong local source tetraalkyllead is most likely to represent 1-4% of total lead in urban air with an overall range of 0.5-8%.

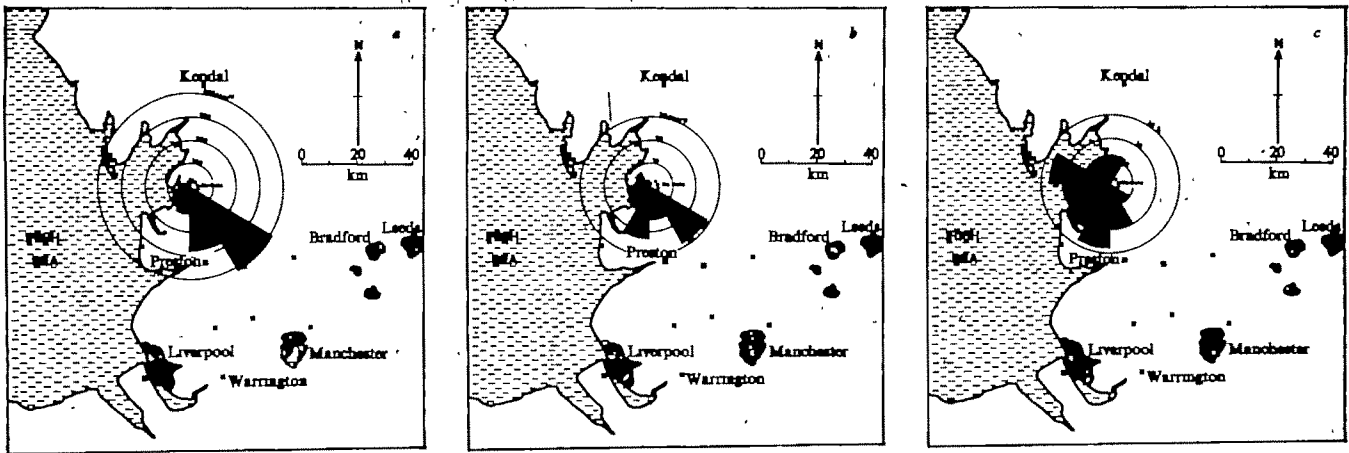
Table 1 Concentrations of particulate lead and tetraalkyllead in urban and rural air

	Particulate lead (ng m ⁻³)	Tetra- alkyllead (ng m ⁻³)	Ratio tetra- alkyllead/ total lead (%)	Source
Urban air (typical values)	500-14,500	<6-206	0.3-4	ref. 10
Petrol station	5,500-6,000	210-590	4-10	ref. 10
Underground car park	10,800-12,200	1,800-2,200	15-18	ref. 10
Rural air	10-660	0.5-230	1.5-33	This work

Lead pollution of rural air arises mainly from advection of urban pollution, and the concentrations of particulate lead and tetraalkyllead in rural air due to urban emissions depend on atmospheric dispersion and dilution effects, the distance of the urban source from the rural site, and the extent of losses *en route*. Only the latter, however, affects the relationship between particulate lead and tetraalkyllead concentrations. It is well known that a large proportion of particulate lead emission is within the size range of particles which form a stable aerosol, so that this lead is lost only slowly from the atmosphere, with a mean lifetime probably in the range 1-7 d.¹² We have determined the mechanism of tetraalkyllead breakdown in the atmosphere¹³ and can predict that tetramethyllead, the predominant tetraalkyllead compound in the atmosphere¹⁰, will have a typical mean lifetime of the order of several days. The loss of both particulate lead and tetraalkyllead from the atmosphere will therefore occur at similar rates, with a resultant ratio of tetraalkyllead to total lead predicted for a rural site of close to the original urban value of 1-4%.

Our data, summarised in Table 1, show that there is a significant departure from expected values. The concentration range for particulate lead, as anticipated, lies considerably below that in urban areas, while the range for tetraalkyllead does not differ so greatly. Indeed, the peak rural concentration of 0.23 µg m⁻³ tetraalkyllead is rarely exceeded in street air measurements¹⁰. Furthermore, seven out of the 28 samples

Fig. 1 Time-weighted pollution roses for lead concentrations measured at three rural sites around Lancaster. a, Particulate lead; b, tetraalkyllead; c, percentage ratio tetraalkyllead/total lead.



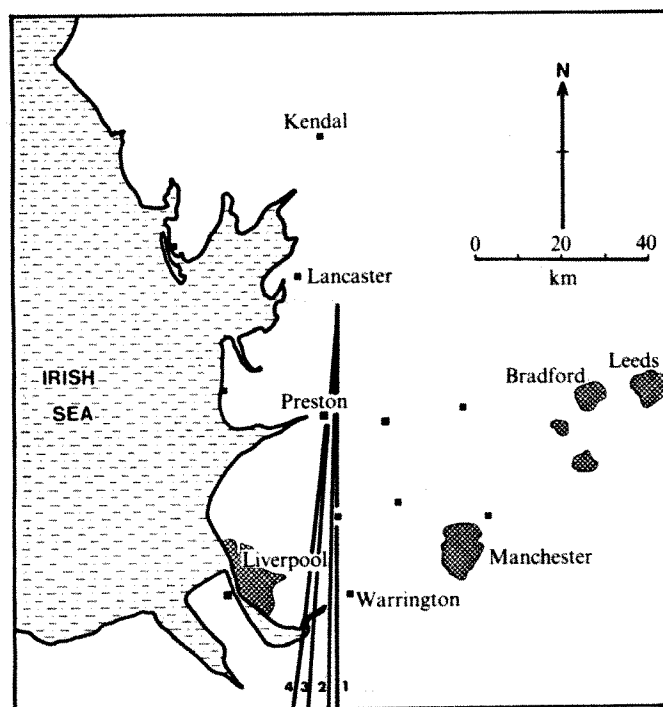
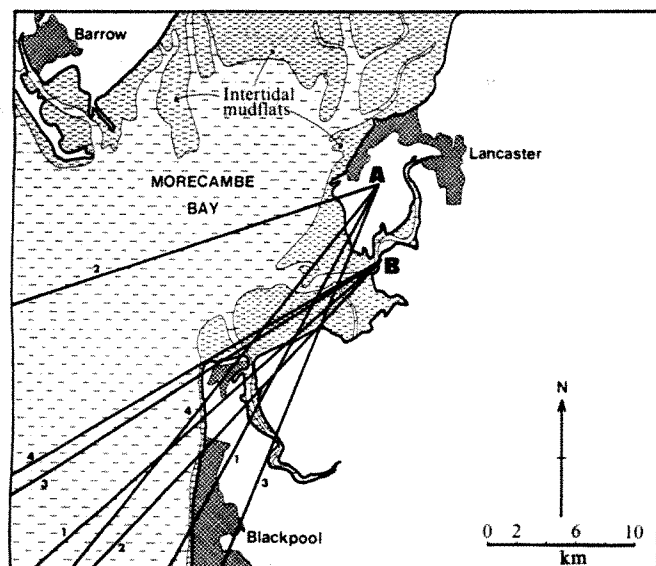


Fig. 2 Terminal directions of geostrophic backward air mass trajectories for a sample period with a tetraalkyllead/total lead ratio of 1.5%. Time of arrival of air mass: 1, 1800 h 18/10/77; 2, 0000 h 19/10/77; 3, 0600 h 19/10/77; 4, 1200 h 19/10/77.

revealed unusually high tetraalkyllead to total lead percentages, in excess of 15%, with a maximum of 33%. Such high percentages are most unlikely to arise from urban emissions. We therefore examined our data in more detail, to identify any relationship between the concentrations and wind direction during a sampling period. Time-weighted pollution roses were constructed, based on hourly wind direction frequency data at 10 m height, divided into 30° sectors (Fig. 1). These reveal the expected pattern for particulate lead, with evidence of the strong influence of urban emissions to the south and south-east of the

Fig. 3 Terminal directions of geostrophic backward air mass trajectories for sample periods with a tetraalkyllead/total lead ratio in excess of 20%. A, Ratio of tetraalkyllead/total lead = 25%. Time of arrival of air mass: 1, 1200 h 30/8/77; 2, 1800 h 30/8/77; 3, 0000 h 31/8/77; 4, 0600 h 31/8/77. B, Ratio of tetraalkyllead/total lead = 27%. Time of arrival of air mass: 1, 1800 h 16/12/77; 2, 0000 h 17/12/77; 3, 0600 h 17/12/77; 4, 1200 h 17/12/77.



sampling sites (Fig. 1a). The picture that emerges for tetraalkyllead, on the other hand, is far less clear (Fig. 1b). When, however, the data for the percentage of tetraalkyllead in total lead are examined a distinct directional influence is revealed (Fig. 1c). High mean sector values of 10–15% are associated with winds of 180–300°, while lower mean values of 5–6% occur with winds from other quarters. These data indicate that a widespread source of tetraalkyllead emissions lies generally to the west of the sampling sites.

Because wind directions indicate only local low-level air movements, geostrophic backward air mass trajectories have also been constructed at 6-h intervals during selected 24-h sample periods (that is, four trajectories per sample), to show synoptic scale air movements. The lowest individual percentage values measured of about 2% are associated with air which had passed over the urban areas to the south and south-east (Fig. 2).

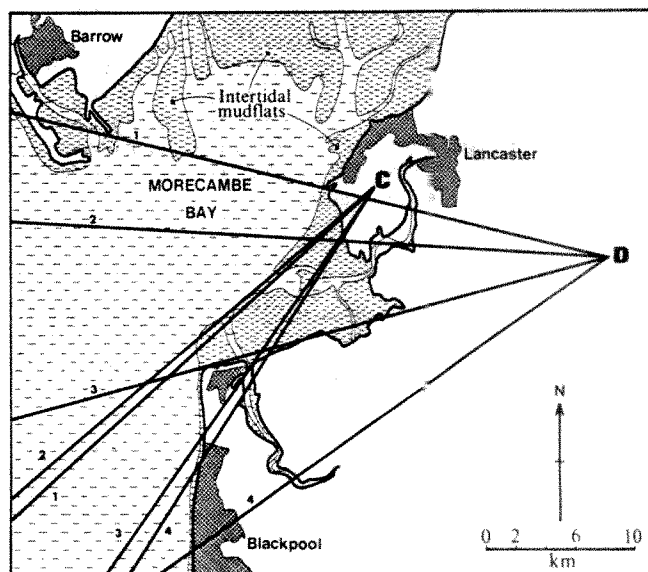


Fig. 4 Terminal directions of geostrophic backward air mass trajectories for sample periods with a tetraalkyllead/total lead ratio in excess of 20%. C, Ratio of tetraalkyllead/total lead = 21%. Time of arrival of air mass: 1, 1200 h 31/8/77; 2, 1800 h 31/8/77; 3, 0000 h 1/9/77; 4, 0600 h 1/9/77. D, Ratio of tetraalkyllead/total lead = 33%. Time of arrival of air mass: 1, 1800 h 31/10/77; 2, 0000 h 1/11/77; 3, 0600 h 1/11/77; 4, 1200 h 1/11/77.

These values are consistent with those to be expected in an urban plume largely unaffected by losses of either particulate lead or tetraalkyllead. On the other hand, periods during which values exceeded 20% are all associated with air which had passed over open sea and the coastal areas adjacent to the sampling sites (Figs 3 and 4). Further examination of our data leads us to suggest that the source of tetraalkyllead is more likely to be associated with the extensive coastal/estuarine intertidal mud flats to the west of our sampling sites, rather than the open sea. Both seawater and sediments in this region have been shown to have elevated levels of inorganic lead (ref. 14 and S. R. Aston personal communication), presumably arising from estuarine discharges of anthropogenic origin. In our view, this source of tetraalkyllead can only be explained by a natural environmental methylation of lead.

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Light-dependent net production of carbon monoxide by plants

PLANTS take up ^{14}CO and incorporate it into organic compounds in the light and in the dark, and it has been suggested that this decreases the concentration of CO polluting the atmosphere^{1,2}. Measurements of ^{14}CO uptake, however, have excluded any concomitant release of CO by the plants and are insufficient for conclusions to be drawn on net CO exchange. Many organisms (microbia, plants, fungi and animals, including man) can produce CO (refs 3-5). We report here that using a Siemens U 180 process gas chromatograph, we have observed a light-dependent net production of CO by intact plant shoots which seems to be linked to photorespiration.

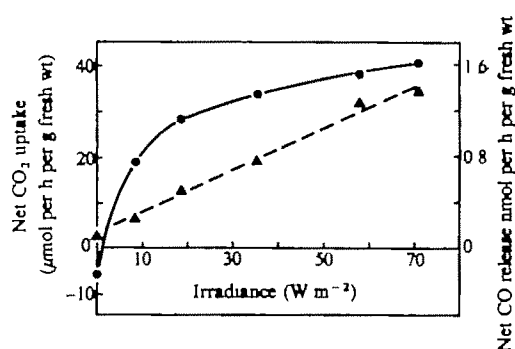


Fig. 1 Dependence of CO (▲) and CO₂ (●) exchange on irradiance. Leaf temperature 28.3 °C; CO concentration in the gas stream supplied to the gas-exchange chamber 2.07 mm³ m⁻³, average CO concentration within the chamber during the experiment 4.78 mm³ m⁻³; CO₂ concentration 250 cm³ m⁻³; O₂ concentration 21 vol %.

In two sets of experiments the shoot of an intact plant of *Nerium oleander* L. with a total leaf surface area of 0.07 m² and 0.18 m², respectively, was sealed in a gas exchange chamber with a volume of 0.01 m³. (A leaf surface of 0.0065 m² is equivalent to 1 g fresh weight. Similar responses are observed with *Vicia faba* L.). Light was obtained from two 400-W HRIL phosphorus-coated metal halide lamps. Various mixtures of gases were passed through the chamber at 30×10^{-6} m³ s⁻¹. Net CO exchange was measured with the gas chromatograph and net CO₂ exchange with an URAS 2T infrared gas analyser. The mixtures were obtained by mixing O₂, N₂, CO₂ and synthetic air from gas cylinders, using dosage pumps and gas flow meters. Traces of CO were removed by passing the gas stream over granulated CO catalyser (Dräger). When required, different concentrations of CO were obtained by adding to the CO-free mixtures various amounts of normal laboratory air whose CO concentration was known from analysis with the gas chromatograph. The atmosphere at our laboratory, at the periphery of the eastern part of Darmstadt, contained up to 3 cm³ m⁻³ CO at peak traffic hours and 0.3 cm³ m⁻³ at other times of the day. This CO caused some problems because of temperature-dependent diffusion of CO into the gas-exchange chamber through small but unavoidable leaks in the apparatus. This diffusion was 1.9 nmol h⁻¹ at 16.6 °C, 3 nmol h⁻¹ at 22.5 °C and 13.8 nmol h⁻¹ at 39.2 °C. Corrections for this contamination were made in calculating net CO production by the plant.

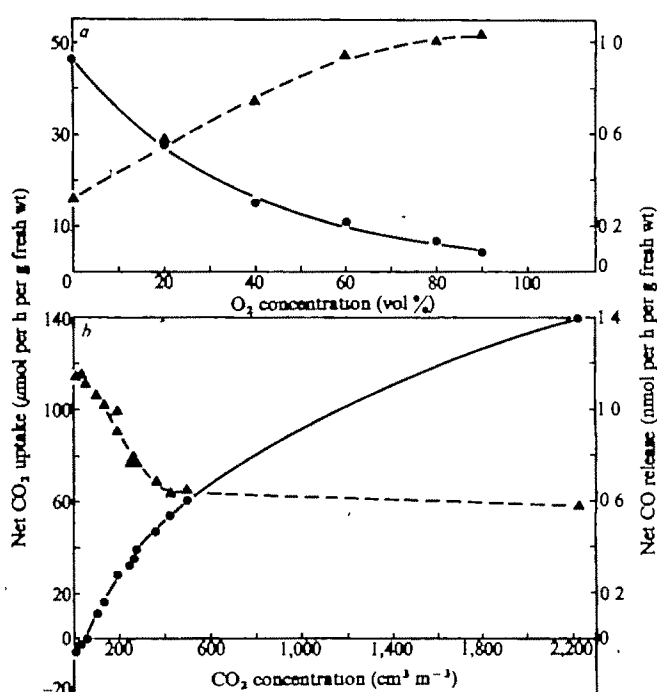
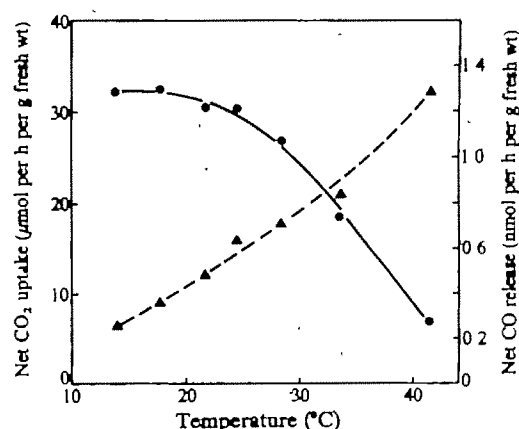


Fig. 2 *a*, Dependence of CO (▲) and CO₂ (●) exchange on O₂ concentration. Leaf temperature 27.5 °C; CO concentration supplied to the gas-exchange chamber 1.61 mm³ m⁻³, average CO concentration within the chamber 4.63 mm³ m⁻³; CO₂ concentration 275 cm³ m⁻³; irradiance 28.0 W m⁻². *b*, Dependence of CO and CO₂ exchange on CO₂ concentration. Leaf temperature 25.5 °C; CO concentration supplied to the gas-exchange chamber 1.9 mm³ m⁻³, average CO concentration within the chamber 4.45 mm³ m⁻³; O₂ concentration 21 vol %; irradiance 35.8 W m⁻².

When the atmosphere in the chamber contained 40–825 mm³ m⁻³ CO the *N. oleander* leaves consistently increased the CO concentration by 10–17 mm³ m⁻³. When the atmosphere contained even 100–120 cm³ m⁻³ CO, plants still did not show a net absorption of CO (ref. 6).

Net production of CO was then studied with low concentrations of CO in the gas stream supplied to the chamber (~2 mm³ m⁻³) and compared with CO₂ gas exchange. CO production was stimulated considerably by light; there seemed to be a small insignificant release of CO in the dark (Fig. 1). Production of CO in the light was increased by increasing the

Fig. 3 Dependence of CO (▲) and CO₂ (●) exchange on air temperature in the gas-exchange chamber. CO concentration supplied to the gas-exchange chamber 2.02 mm³ m⁻³, average CO concentration within the chamber 4.54 mm³ m⁻³; CO₂ concentration 250 cm³ m⁻³; O₂ concentration 21 vol %; irradiance 18.7 W m⁻².



concentration of O_2 and decreased by increasing the concentration of CO_2 in the ambient atmosphere (Fig. 2). Photosynthetic CO_2 fixation showed an inverse relationship to the concentrations of CO_2 and O_2 (Fig. 2). Increasing temperature greatly enhanced CO release (Fig. 3).

As photorespiration responds in a similar way to environmental parameters⁷⁻⁹, these results suggest that light-dependent production of CO is a by-product of photorespiratory C_1 metabolism. CO could be derived from the glycolate pathway of photorespiration in two ways. First, direct oxidative decarboxylation of glycolate may produce formate¹⁰ and hence CO . Second, formate and CO may originate from the tetrahydrofolate activated C_1 unit formed during photorespiratory synthesis of one molecule of serine from two molecules of glycine¹¹. The significance of this minimal production of CO is not clear; nonetheless, the net exchange of CO by plants is a small emission, not absorption.

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Photorespiratory nitrogen cycle

MOST temperate plants form phosphoglyceric acid as the first stable product of photosynthesis. These plants also photorespire, that is, during CO_2 fixation they simultaneously release CO_2 to the atmosphere from the oxidation of an intermediate of the photosynthetic process^{1,2}. The reaction below, which takes place in the mitochondria^{3,4}, is chiefly responsible for this CO_2 release.



Photorespiration is considered wasteful because it loses CO_2 and energy, but little emphasis has been placed on the simultaneous and stoichiometric release of NH_3 . Based on estimates of photorespiration in wheat leaves of $80 \mu\text{mol } CO_2 \text{ h}^{-1} \text{ g}^{-1}$ fresh weight^{5,6}, NH_3 loss far exceeds the calculated rates of primary NH_3 assimilation from NO_3^- reduction⁷⁻⁹. This NH_3 must be reassimilated if the plant is not to lose all its organic nitrogen. Recent ^{15}N studies show that NH_3 is recycled¹⁰ but it is not known if reassimilation is catalysed by glutamine synthetase (GS), as is primary NH_3 assimilation^{11,12}, or by glutamate dehydrogenase (GDH) also present in the mitochondria^{11,12}. Identification of the pathway is important because it determines the extent to which NH_3 cycling further increases the energy loss caused by photorespiration. We report here experiments which show that reassimilation involves GS and we propose a series of reactions which make up the photorespiratory nitrogen cycle.

An estimate of the amount of GS and GDH in wheat leaves was made. The values obtained, in nKat per g fresh weight, of 40 for GS and of 54 for GDH indicate that both enzymes may be present in sufficient amounts to catalyse NH_3 reassimilation despite the high K_m of GDH for NH_3 .

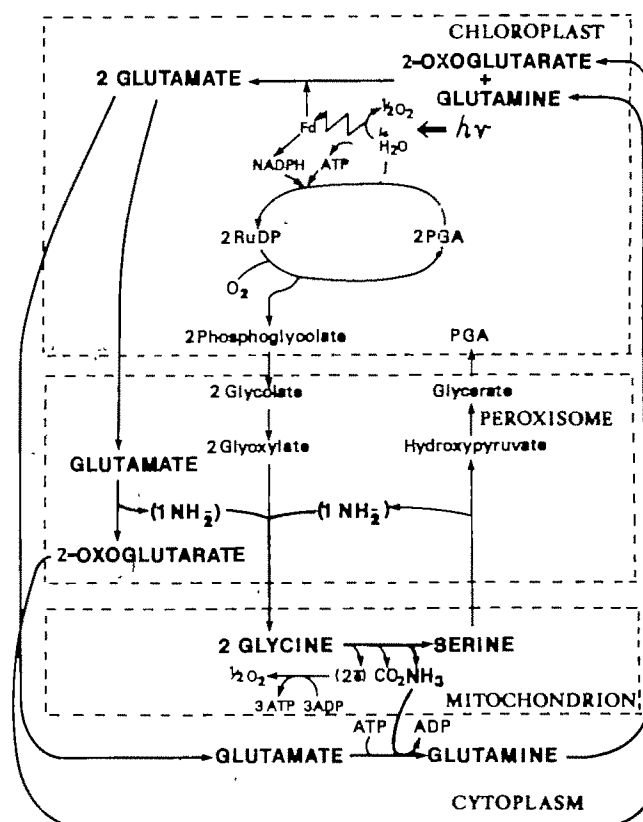


Fig. 1 The photorespiratory nitrogen cycle (the complex stoichiometry of the Calvin cycle is not included).

To study the loss of NH_3 and its reassimilation *in vitro*, mitochondria were isolated from spinach leaves by the method of Douce *et al.*¹³. They were shown to produce $^{14}\text{CO}_2$ and $^{15}\text{NH}_3$ in about equal amounts from [^{15}N , 1- ^{14}C]-glycine (Table 1); serine was also produced as found previously with tobacco³ and spinach¹⁴ mitochondria. The mitochondrial preparations contained substantial amounts of GDH (about a third of the total in the original homogenate) and small amounts of GS ($\leq 0.2\%$); this GS was separated from the mitochondrial marker enzymes when the preparations were centrifuged on a sucrose density gradient. To test if the NH_3 produced by the mitochondria could be reassimilated *in situ* the various reaction components required for GDH or GS activity were added to the mitochondrial incubations (Table 1, treatments 2-4); there was no decrease in the amount of NH_3 evolved or in the ratio of $^{15}\text{NH}_3/^{14}\text{CO}_2$ released. These results, and those of Woo and Osmond¹⁴ showing that NH_3 is not retained within the mitochondria, suggest that reassimilation occurs outside.

Recent experiments have shown that GS is almost equally distributed between the chloroplast and the cytosol¹⁵; the cytosol GS, present immediately external to the mitochondria, might well function in reassimilation. To test this, chloroplasts were removed from an homogenate of pea leaves by centrifugation¹⁶. The GS was purified 100-fold from the supernatant liquid by ammonium sulphate precipitation and sequential chromatography on Sephadex G-25, DEAE-cellulose and Sepharose 4-B. Preparations, with a minimum specific activity of 111 nKat per mg protein as measured by the γ -glutamyl synthetase assay¹⁷, were added to reaction mixtures also containing mitochondria. Difficulties were experienced in combining the two systems, as the requirements of each for maximum activity differ—the conditions indicated in Table 1 represent a compromise. In the presence of GS little NH_3 was recovered from the reaction medium, indicating that it had been reassimilated, but there was increased release of $^{14}\text{CO}_2$ and the ratio of $^{15}\text{NH}_3/^{14}\text{CO}_2$ dropped to 0.13. The increase in CO_2 release could be explained by a faster rate of electron transport

Table 1 Release of NH_3 and CO_2 from [^{14}C , ^{15}N]-glycine by mitochondria

Treatment	Reaction medium	CO_2 released from glycine (μmol)	NH_3 released from glycine (μmol)	Total NH_3 released (μmol)	Ratio of $^{15}\text{NH}_3$: $^{14}\text{CO}_2$
1	Control	0.623	0.633	0.870	1.01
2	Control + 2-oxoglutarate	0.621		0.875	
3	Control + glutamate	0.615		0.945	
4	Control + 2-oxoglutarate, glutamate and oxaloacetate	0.847	0.915	1.182	1.08
5	Control	1.04	1.15	1.31	1.10
6	Control + GS	1.85	0.24	0.35	0.13

Treatments 1–4 control contained, 3.3 mM ATP, 16.4 mM (1- ^{14}C , ^{15}N)-glycine (0.05 mCi per mmol 99 atom % ^{15}N), 246 mM mannitol, 4.1 mM MgCl_2 , 8.2 mM KCl, 8.2 mM KH_2PO_4 , 0.08% bovine serum albumin, mitochondria from market spinach containing 0.8 mg protein and, where indicated, 16.4 mM glutamate, 16.4 mM 2-oxoglutarate, 16.4 mM oxaloacetate. The final volume (0.61 ml) was incubated at pH 7.2 for 20 min at 22 °C. Treatments 5–6 control contained, 4 mM ATP, 40 mM glutamate, 5 mM KH_2PO_4 , 10 mM KCl, 12 mM MgCl_2 , 10 mM MOPS, 10 mM Tris, 0.3 M mannitol, 0.2% bovine serum albumin, mitochondria from glasshouse spinach containing 0.4 mg protein and, where indicated, 2.5 nKat of glutamine synthetase. The final volume (0.60 ml) was incubated at pH 7.8 for 30 min at 22 °C. The reaction was stopped by addition of an equal volume of $\text{C}_2\text{H}_5\text{OH} \cdot \text{NH}_3$ was distilled isothermally at 20 °C into 0.1 ml 1 M H_2SO_4 in an evacuated flask after buffering the mixture at pH 10. The acid solution of $(\text{NH}_4)_2\text{SO}_4$ was mixed with 9 ml NH_3 -free H_2O , 0.1 ml 2 M NaOH and 0.2 ml 10% (w/v) sodium potassium tartrate and the tubes incubated at 25 °C for 10 min. Nessler's reagent (0.4 ml) (ref. 21) was added and after 30 min at 25 °C absorbance at 435 nm was measured. The Nessler complex was precipitated by adding four pellets NaOH (0.4 g) to each tube, mixing, and storing in the dark for 30 min. The precipitate was washed with 0.5 ml NH_3 -free H_2O and suspended in 40 μl for each μmol of NH_3 of 1 M HCl in 10% aqueous $\text{C}_2\text{H}_5\text{OH}$ (ref. 22). After standing overnight at 0–5 °C the insoluble material was removed by centrifugation and 8- μl samples of the supernatant were dried in short lengths of capillary tube at 60 °C. The residues were sealed *in vacuo* (0.2 Pa) in a pyrex tube with appropriate reagents and the ^{15}N , ^{14}N content measured by emission spectroscopy²³. Radioactive serine and CO_2 were removed and measured as described previously³.

resulting from the production of ADP from ATP by GS. This was confirmed in oxygen electrode experiments with the same batch of mitochondria.

The above results indicate that reassimilation can occur *in vitro* with GS. To confirm that this also occurs *in vivo* carefully matched flag leaves of wheat (c.v. Sicco) were detached under water, stood vertically with their bases in 3 ml of water and illuminated. Such leaves contained small amounts of NH_3 which increased when they were supplied with ^{15}N -glycine by the cut

base (Table 2). Measurement of the ^{15}N content of the NH_3 indicated that the extra amount was derived from glycine. The addition of methionine sulfoximine (MSO) together with the ^{15}N -glycine increased the amount of NH_3 derived from glycine 4.5-fold. Also, when isolated pea protoplasts were incubated with ^{15}N -glycine, MSO doubled the production of $^{15}\text{NH}_3$. There was, however, a considerable background of $^{14}\text{NH}_3$ production by the protoplasts. A similar release of NH_3 from freshly isolated poppy cells has been observed¹⁸. As MSO is a specific inhibitor of GS¹⁹, we conclude that NH_3 produced during the conversion of glycine to serine *in vivo* is normally reassimilated by GS.

On the basis of the above results we propose that photorespiration involves a cycling of nitrogen (see Fig. 1). If the generally accepted estimates of photorespiration and primary assimilation of NH_3 derived from nitrate reduction are correct, then the flux of NH_3 through the photorespiratory nitrogen cycle is an order of magnitude greater than that resulting from primary assimilation. In the scheme, the NH_3 released from glycine is refixed by cytoplasmic GS into glutamine. Glutamine and 2-oxoglutarate move into the chloroplast where, in the presence of reduced ferredoxin and glutamate synthase, two molecules of glutamate are formed; one of these molecules of glutamate becomes the acceptor molecule for another NH_3 molecule and the other provides a transamination equivalent to convert one glyoxylate to glycine while regenerating 2-oxoglutarate (a second transamination equivalent is needed and comes from serine).

As chloroplasts actively assimilate ammonia in the light²⁰, it is possible that chloroplastic GS could be partly involved, but ammonia produced in mitochondria probably first encounters the cytoplasmic GS. The conversion of glutamine to glutamate must occur solely in the chloroplast as that is the only site of glutamate synthase activity in the leaf cell¹⁵. The operation of the cycle would minimise the energy required in the cytoplasm, as the major energy-demanding step is directly coupled to the light-dependent reduction of ferredoxin. Thus, only one ATP of the three generated in the mitochondria during the conversion of two molecules of glycine to serine^{3,4,13} is needed for reassimilation of ammonia. The two molecules of ATP remaining may be used for synthetic reactions in the cytoplasm.

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Table 2 Effect of methionine sulfoximine on NH_3 release from [^{15}N]-glycine

Solution supplied	Time (min)	Total NH_3 released (μmol)	NH_3 released from glycine (μmol)
Wheat leaves			
Water	45	1.7	—
	90	1.4	—
5 mM MSO	45	5.8	—
	90	12.0	—
15 mM ^{15}N -glycine	45	2.5	0.6
	90	3.5	1.4
15 mM ^{15}N -glycine + 5 mM MSO	45	8.9	2.7
	90	17.4	6.4
Pea leaf protoplasts			
^{15}N -glycine + glutamate	60	0.45	0.08
^{15}N -glycine + glutamate + MSO	60	0.68	0.16

Wheat flag leaves, each about 0.45 g fresh weight were illuminated for 40 min at 25 °C with the cut bases in water. The water was then replaced by the solutions indicated. The photon flux density at the leaf surface was 800 $\mu\text{Einstein m}^{-2} \text{s}^{-1}$ (PAR). Pea leaf protoplasts were isolated by the method of Wallsgrove *et al.*¹⁵ and an amount containing 207 μg chlorophyll incubated at 25 °C in 0.59 ml containing 16.95 mM [^{15}N]-glycine (99 atom % ^{15}N), 16.95 mM glutamate, 0.34 M sorbitol, 80 μM MgCl_2 , 42 mM Tricine pH 7.5 and, where indicated, 1.93 mM MSO. The reaction mixture was illuminated with a photon flux density of 1,200 $\mu\text{Einstein m}^{-2} \text{s}^{-1}$ (PAR) in tubes gassed with air at 300 ml min^{-1} and shaken at 1 Hz. Wheat leaves were killed in boiling H_2O and homogenised; reaction mixtures containing protoplasts were treated with an equal volume of $\text{C}_2\text{H}_5\text{OH}$. Measurements of the amount and ^{15}N content of NH_3 were as in Table 1.

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Bats avoid moving objects more successfully than stationary ones

MICROCHIROPTERANS sense their environment by emitting ultrasonic signals and listening to the echoes¹. The simplest way to measure this ability is by the obstacle-avoidance test²⁻⁷, in which a bat flies between an array of stationary wires and the numbers of hits and misses are recorded. We have extended this test and made our bats fly between an array of moving as well as stationary obstacles. We were surprised to find, as reported here, that a bat can avoid moving obstacles more successfully than stationary ones.

Six *Myotis lucifugus* (the little brown bat) with an average maximum wingspan of 23 cm were tested in a room 4.6 m long, 2.2 m wide and 2.1 m high. A wooden frame containing six vertically strung nylon monofilament lines or steel wires (the obstacles) was placed in the centre of the room. The total opening of this barrier (the frame plus the obstacles) through which a bat flew was 1.8 m wide and 1.7 m high. By means of an electric motor the barrier was made to oscillate in the horizontal direction parallel to the alignment of the obstacles, with a maximum amplitude of 9 cm at a rate of 0.39 Hz, which

generated an average speed of 7 cm s⁻¹. Any effect of the movement of the frame itself on the bat's performance was eliminated by surrounding the frame with large wooden panels which were attached to the walls, ceiling and floor. Thus the bat could see the movement of the lines or wires, but not that of the frame. The sound of the moving lines or wires was negligible. All surfaces of the wooden panels, ceiling and walls were covered with convoluted polyurethane foam to reduce the echoes from them. Four different diameters of nylon lines (0.23, 0.37, 0.47 and 0.53 mm) or steel wires (0.32, 0.37, 0.53 and 1.53 mm) were used, one size at a time for each test. The distance between the obstacles was always 30 cm. A bat was made to fly between stationary obstacles at least 32 times before it was made to fly between moving ones another 32 times in order to minimise any effect of spatial memory of the position of the obstacles^{8,9}. Each bat was tested in this way as many times as possible. A hit consisted of collisions of the body or arm with the obstacles. A miss consisted of flights in which the obstacles were not touched or were lightly brushed by a wingtip with no change of flight course.

When an agile bat flew between the stationary obstacles, it negotiated them almost without hesitation. However, when the barrier was moved, the bat hovered first at one side of the flight room before flying between the obstacles. The bat was apparently more cautious when confronted with moving obstacles. Bats tended to fly between the upper central part of the barrier regardless of whether the obstacles were stationary or moving.

Contrary to our prediction, the percentage of misses by all bats was always greater for the moving obstacles than for the stationary ones, regardless of the size of the obstacles. Table 1 summarises the data for four bats which were tested with at least two different diameters of wires or lines. The average percentage of misses obtained in each of the two test conditions differed by 6.5-15%. All these differences are significant (χ^2 test, $P < 0.10$).

Myotis lucifugus flies between an array of obstacles of this sort with an average wingspan of 14 cm (60% of maximum wingspan)^{1,2}. Therefore a model bat with a wingspan of 14 cm was made from wooden sticks, steel wires and tapes. It was thrown at random, in the manner described by Hahn⁶, towards the barrier in order to find out the chance of missing the barrier. Six diameters of nylon line (0.20, 0.27, 0.33, 0.56, 0.83 and 1.0 mm) were used; Table 1 shows only the data obtained with 0.20, 0.56 and 1.0-mm lines. In general, the percentage of misses when the obstacles were moving was not significantly different from that recorded when the obstacles were stationary, regardless of the diameters of the lines. The average percentage of misses for stationary and moving obstacles of six different diameters was 65.2 and 65.4, respectively. These values are 30-40% greater than those reported earlier^{1,2,6}. Because the percentage of misses by bats obtained in either test condition was significantly greater than those found with the model bat, the bats apparently used echolocation to dodge the obstacles.

Table 1 Avoidance of obstacles by bats

Diameters of wires (w) or lines (l) (mm)	Bats tested	Stationary		Moving		Differences in average % misses	χ^2	Significance at 90% correct level $P \leq 0.10$
		Total flights	Average % misses	Total flights	Average % misses			
0.32 (w)	No. 1 & 2	256	72	256	79	7	3.4	Yes
0.37 (w)	No. 1 & 2	352	78.5	320	85	6.5	4.8	Yes
0.53 (w)	No. 1 & 2	256	81.5	220	89	7.5	5.2	Yes
1.53 (w)	No. 1 & 2	192	79.5	192	88	8.5	4.9	Yes
0.23 (l)	No. 3	192	76	192	91	15	16.0	Yes
0.37 (l)	No. 3 & 4	288	80.5	288	91.5	11	14.9	Yes
0.47 (l)	No. 3 & 4	320	80.5	320	90.5	10	13.0	Yes
0.53 (l)	No. 3	128	86	128	93	7	3.4	Yes
0.20 (l)	Model	120	65	120	67.5	2.5	0.2	No
0.56 (l)	Model	128	65	123	70	5	0.7	No
1.0 (l)	Model	323	67	192	64	3	0.4	No

Why was a moving obstacle easier to miss (or perceive) than a stationary one? The answer is probably associated with the fact that carnivorous and insectivorous bats rely on moving prey for survival, and they apparently concentrate more intently on moving objects while hunting. In behavioural experiments, it is essential to present an animal with tasks which are more relevant to situations encountered in its natural environment.

'Motion detectors' have been found in a cat's inferior colliculus¹⁰ and auditory cortex¹¹, and it is likely that the bat's auditory system also contains neurones that are particularly sensitive to an echo source moving in a particular direction and at a particular speed. Indeed, cortical neurones sensitive to a moving sound source have been found in the big brown bat (*Eptesicus fuscus*) by P. Wasserbach (personal communication).

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Pygmy chimpanzee as a possible prototype for the common ancestor of humans, chimpanzees and gorillas

A CONVINCING theory of human origins must clarify man's relationships with living primates and with the ancestral forms known only through fossils. Phylogenetic relationships have previously been determined mainly by anatomical similarities, but now, biochemical similarities provide independent criteria for evolutionary relationships. Albumin and transferrin immunology, immunodiffusion, DNA annealing and amino acid analysis all indicate that chimpanzees, gorillas and humans share a substantial common ancestry, and that the Asiatic apes (gibbons and orangutans) diverged earlier from this lineage¹⁻³. These findings directly conflict with the more widely held view that all the great apes diverged from a common ancestor long after the 'origin' of the evolutionary line leading to modern humans⁴. The molecular data consistently suggest a much more recent origin of the man-chimpanzee-gorilla separation than was previously imagined, namely, in the range of 4-6 M yr ago^{3,5}. These data show that, although the two chimpanzee species (*Pan paniscus* and *P. troglodytes*) are biochemically distinct, they are more closely related to each other than either is to humans or gorillas^{6,7}. The chimpanzees speciated, then, after the initial three-way split. We therefore, here contend that, among living species, the pygmy chimpanzee (*P. paniscus*) offers us the best prototype of the prehominid ancestor. Biochemical, morphological, behavioural and palaeontological data support this proposition and argue for a relatively recent and accelerated divergence of the hominid from the pongid line.

If this cladogram is correct, then an ancestor for the earliest hominids must also be a suitable ancestor for chimpanzees and

gorillas. The morphological similarities between the three groups should logically be derived from the common ancestor, rather than by evolutionary convergence, or by parallelism from distinct primate lineages. In fact, the modern African apes may be viewed as size variants in a single morphotypic series, going stepwise from the smaller *P. paniscus* to the large male gorilla. The scant hominid fossil record during the past 4-8 M yr, and the absence of any fossil chimpanzees or gorillas, forces us to work backwards from the living hominoids to reconstruct a prototype of the common ancestor.

Chimpanzees have generally been considered closer to this prototypic ape than gorillas, because the latter are more specialised morphologically and behaviourally. They are larger and males are twice the size of females; they have a more specialised diet, more restricted habitat and less flexible social behaviour than chimpanzees. Morphologically, pygmy chimpanzees are more 'generalised' in body size, body build and sexual dimorphism than the common species and may provide a better prototype for studying the derivation of the earliest hominids and African apes.

Pygmy chimpanzees on the average weigh less than the average common chimpanzee, but with much overlap: 25-48 kg, compared to 25-60 kg or somewhat more⁸. Pygmy chimpanzees have smaller facial and canine dimensions, smaller average cranial capacities (350 cm³ compared with 390 cm³), and the two species can be discriminated on mandibular length alone⁹.

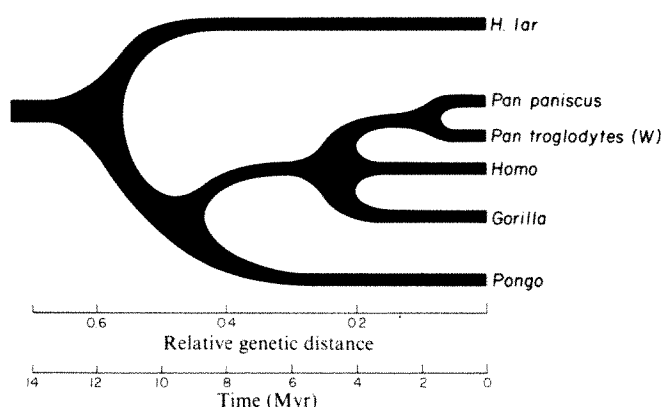


Fig. 1 Molecular cladogram showing genetic relationships of the ape and human lineages.

Pygmy chimpanzees have a narrower trunk and more gracile upper body, as reflected in significantly smaller clavicular length, scapular dimensions and iliac and sacral breadths⁸. Their upper and lower limbs are of about equal length, whereas arms of common chimpanzees are relatively longer.

As to sexual dimorphism, pygmy chimpanzees are moderately sexually dimorphic in body weight, but no dimorphism can be detected in cranial capacity, limb bone lengths or robusticity, or in anterior and posterior dentition¹⁰. They have relatively small and only slightly dimorphic canine teeth¹¹. Overall, this is less dimorphism than occurs in common chimpanzees and no more than occurs in modern humans.

Observations on the behaviour of pygmy chimpanzees, either in their natural habitat in the Zaire River Basin or in captivity, have not been extensive. They feed high in trees, move and feed on the ground^{12,13}, and in captivity walk bipedally more than do common chimpanzees (S. Savage-Rumbaugh, personal communication). They therefore encompass the spectrum of locomotor behaviour of living African hominoids: arboreality, terrestriality and bipedality. Laboratory and field studies reveal pygmy chimpanzees as highly intelligent and flexible in feeding, locomotor, sexual and communicatory behaviour¹⁴⁻¹⁶.

Given these morphological and behavioural data, we maintain that pygmy chimpanzees present a general pattern from

Table 1 Comparison of pygmy chimpanzee and early hominid pelvis, femur and humerus (in mm)

Measurement	Early hominids	Pygmy chimpanzees
Femur length	280*, 280†	293 (264–315)
Femoral head diameter	31†	30.5 (28–34)
Acetabular diameter	37†	36 (32–38)
Innominate length	170†	253 (232–272)
Iliac breadth	113†	97 (80–118)
Sacral breadth	76†	63 (51–70)
Humerus length	235*	285 (250–307)

* AL 288–1 from Hadar, Ethiopia¹⁸.

† Sts 14 from Sterkfontein, South Africa. Measurements from A.L.Z. (unpublished data); femur length from Lovejoy²³.

which other African hominoids could have developed. This contention is further supported by comparing pygmy chimpanzees with the earliest hominids.

The fossil record, based on dentition, shows the occurrence of *Australopithecus* by 3.5 M yr ago in the mosaic savannas of Eastern Africa¹⁷. By 3 M yr ago, the hominids were distinct from apes in the broader and shorter pelvis, larger cranial capacity, larger postcanine teeth and somewhat smaller canines^{18,19}.

P. paniscus provides a suitable comparison for *Australopithecus*²⁰; they are similar in body size, postcranial dimensions and, as Figs 2 and 3 show, even in cranial and facial features, although this *Australopithecus* has a larger cranial capacity (485 cm³)²¹ than *P. paniscus* (350 cm³).

Postcranial dimensions are given in Table 1. Femoral length, femoral head and acetabular diameters are very similar. Humerus and innominate lengths, and breadths of sacrum and ilium are different. These findings demonstrate early hominid affinities with apes. Probably the initial acquisition of hominid upright locomotion entailed reduction of upper limb length and broadening of the ilium and sacrum.

Homo and *Pan* (both species) differ in their genomes by approximately 1%, a value characteristic of sibling species²². The genetic changes accounting for the morphological differences between man and chimpanzee may be only a small fraction of that amount. A few regulatory genes may have large somatic effects, whereas the remaining structural genes may be time dependent in their rate of fixation, and of little selective significance.

Fig. 2 Side view, drawn to scale, of Sterkfontein 5 (above) and adult female *P. paniscus* (below).

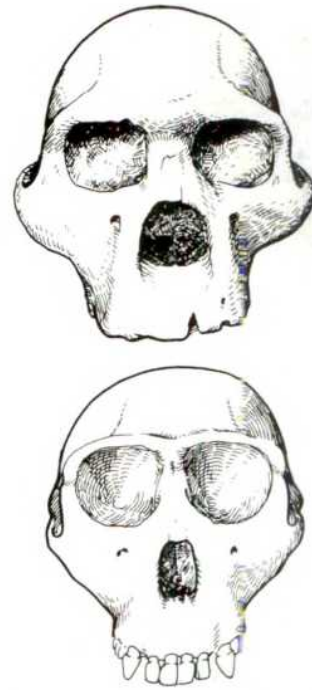
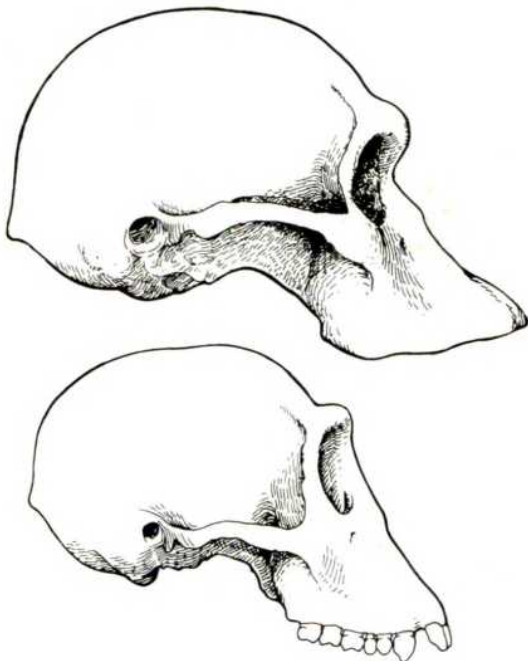


Fig. 3 Front view, drawn to scale, of Sterkfontein 5 and adult female *P. paniscus*.

The ultimate strength of any hypothesis rests on its compatibility with all lines of evidence, on its ability to withstand falsification and on its power of prediction. We hypothesise (1) that as our knowledge of the fossil record improves, hominids before 3.0 M-yr ago should continue to converge on the pygmy chimpanzee-like condition, cranially and postcranially, as they seem to do dentally, (2) that Pliocene pongids ancestral to chimpanzees and gorillas should do likewise, and (3) that at least one lineage of late Miocene ape (perhaps *Ramapithecus*) should continue to develop evolutionary trends begun in the early Miocene and evolve into a form similar to the extant pygmy chimpanzee.

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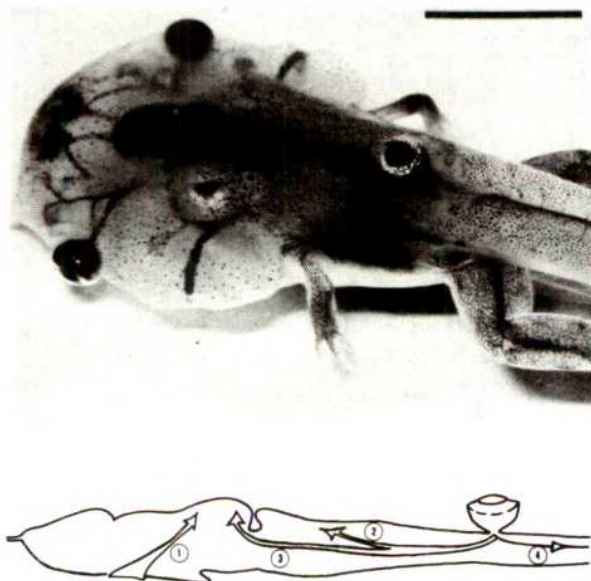
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Axons from eyes grafted in *Xenopus* can grow into the spinal cord and reach the optic tectum

THE transplantation of embryonic eyes in Amphibia is a long established approach for testing the ability of nerve fibres to grow through unusual regions of the central nervous system^{1,2}. As the behaviour of growing ectopic fibres may disclose the mechanisms of axonal guidance³⁻⁷, this approach has recently

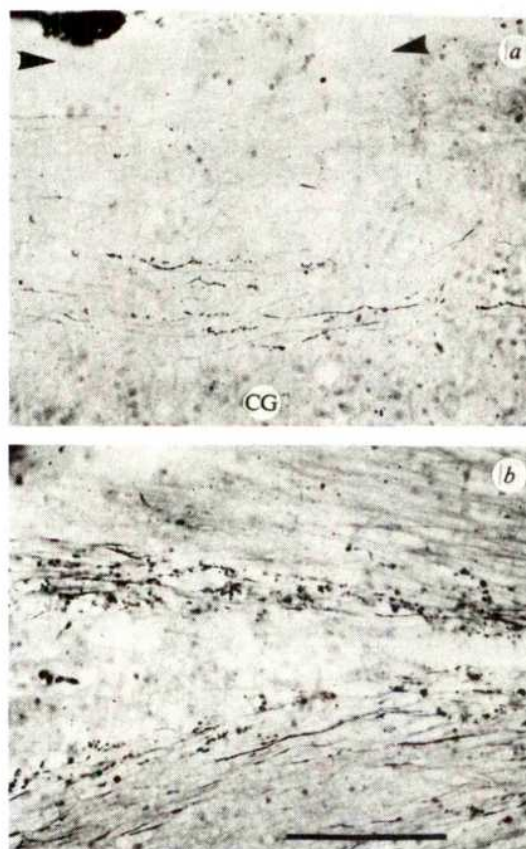
Fig. 1 Tadpole of *Xenopus laevis* with a third eye grafted at embryonic stage 23 (ref. 13) and now (stage 58) growing above the spinal cord. The brain and the normal eyes with their optic nerves can be seen through the transparent tissue of the tadpole head. Embryos were obtained in our laboratory by induced mating¹⁴ of wild animals and were operated on in full-strength Niu and Twitty solution¹⁵ containing antibiotics (10,000 U l⁻¹ of penicillin G and 25 mg l⁻¹ of streptomycin sulphate) and anaesthetic (0.02% MS-222, Sandoz). In the sagittal silhouette of the tadpole CNS the paths of optic fibres issuing from the normal (1) and grafted eyes (2-4) are represented. (1) Normal optic tract terminating in the anterior optic tectum; (2) ectopic fibres terminating in the medulla oblongata in triocular and in blinded (that is, binocularly enucleated) animals; (3) ectopic fibres terminating in the posterior optic tectum in blinded animals; (4) ectopic fibres growing caudally. Scale bar (applies to photograph only), 1 cm.



attracted new interest⁸⁻¹². We are studying the behaviour of optic fibres entering the spinal cord from a heterotopic eye grafted on to the dorsal trunk region of *Xenopus* embryos^{10,11}. Here we report on the paths used by ectopic fibres to travel in the spinal cord, their possible termination in the hindbrain, and their ability to reach the optic tectum.

Eye vesicles from *Xenopus laevis* embryos at stage 25 (ref. 13) were grafted on to the dorsal region of other embryos at stage 23. Results were studied at larval stages. In 52 tadpoles, the grafts developed into morphologically normal eyes located on the back of the animal, just dorsal to the spinal cord and at different distances from the brain (Fig. 1). Twenty animals (out of the 52 mentioned above) were cut sagittally and stained by Holmes' silver method¹⁶ for histological analysis: seven of the grafted eyes had developed an optic nerve whose fibres entered the spinal cord. These fibres seemed to grow mainly rostrally. We have confirmed this by tracing the paths of degenerating fibres in the remaining 32 tadpoles. Twelve of these animals had a considerable number of degenerating fibres in the spinal cord (Fig. 2). In each case we found most argyrophilic debris rostral to the graft; a smaller amount occurred caudally. Quantification of fibres in this type of preparation is not possible, but we estimate that approximately three-quarters of the fibres grew rostrally. No specific path seems to have been used by these optic axons; degenerating fibres were broadly distributed in the dorsal half of the white matter of the spinal cord (Fig. 2a). In particular, they

Fig. 2 Degenerating fibres in the spinal cord of experimental tadpoles. Horizontal section; rostral is left. The grafted eye was opened and the retina was gently peeled out. After 24 h the animals were fixed in neutralised 10% formalin and processed to demonstrate degenerating nerve fibres and terminals by a modification of Nauta's reduced silver method¹⁷. a, The ectopic fibres are localised near the central grey (CG); they were found bilaterally in various positions inside the dorsal white matter, but never close to the pial surface (between arrows). b, Degenerating fibres in the dorsal funiculi; in this region (hindbrain), they are diverging. Scale bar, 100 μ m.



were not grouped near the dorso-lateral surface of the spinal cord, as described by others^{9,12}. In two cases a large proportion of degenerating fibres was concentrated in the dorsal funiculus (Fig. 2*b*). In all cases they occurred bilaterally.

The possible termination of ectopic fibres was studied in the same set of 32 animals mentioned above. In an initial series of 17

12 h after eye transplantation and 15 h before outgrowth of optic fibres¹³. We processed the tadpoles (stages 52–57) to trace the ectopic fibres. In this second series (the remaining 15 tadpoles of the 32 mentioned above), we found three cases with extensive terminal degeneration in the optic tectum, as well as two cases with a weaker tectal projection from the ectopic eye. Terminal degeneration was bilateral and diffuse in the superficial layer of the posterior part of the optic tectum (Fig. 4*a*) and, ventrally, near the nucleus isthmi. In these five cases, bilateral terminal degeneration was also found in the solitary tract.

Sharma⁸ and Constantine-Paton and Capranica⁹ have grafted eyes in *Rana* on to the dorsal diencephalon and the internal ear region, respectively. Their results led to the suggestion⁹ that optic fibres are inherently specified for a dorso-caudal growth, relative to the overall polarity of the brain. The extensive growth of ectopic fibres in a rostral direction along the spinal cord, previously reported by us¹¹ and by Katz and Lasek¹², does not agree with this suggestion. The varied location of degenerating fibres along the spinal cord described here does not support the idea that axons from grafted eyes are growing 'in a specific set of

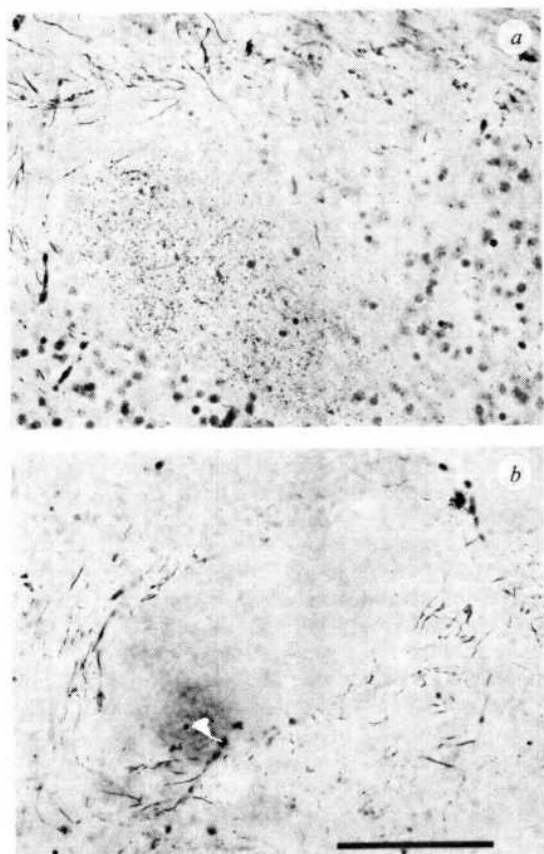
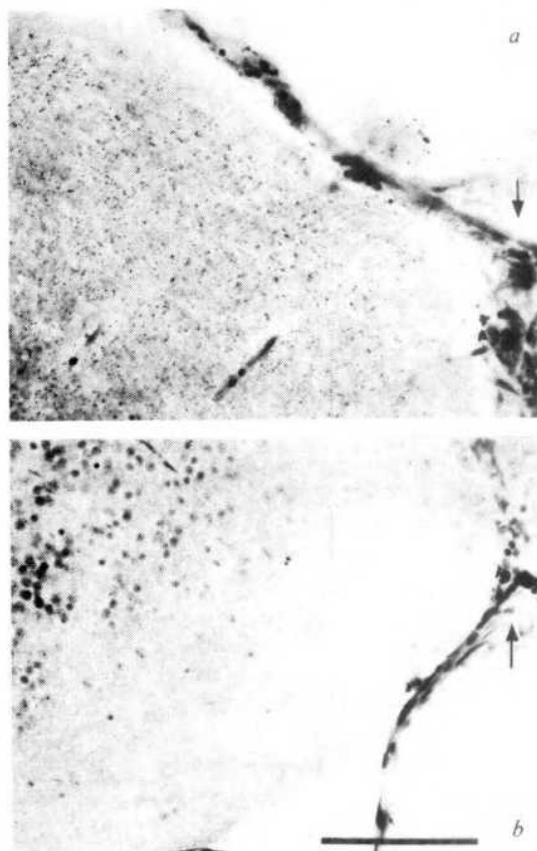


Fig. 3 *a*, Same experimental situation as Fig. 2. Horizontal section; rostral is left. Terminal degeneration is inside the right solitary tract (the phenomenon was bilateral). The nucleus of the solitary tract is not clearly delimited in Amphibia; neurones surrounding the solitary tract send their dendrites into it to receive the terminals from the tract¹⁸. Lateral to the solitary tract (top right) are some degenerating fibres. *b*, Left solitary tract of a control animal in which the dorsal spinal cord was sectioned 24 h before death. No terminal degeneration is present. Scale bar, 100 μ m.

tadpoles with a third eye grown on their back, we found four animals (stages 52–57) with evidence of terminal degeneration in the hindbrain (in three tadpoles there was no terminal degeneration in spite of the presence of optic fibres in the spinal cord). The terminal degeneration was restricted to the 'nucleus' of the solitary tract (Fig. 3*a*)—always occurring bilaterally and along its entire extent—with the exception of one case showing weak terminal degeneration lateral to the nucleus isthmi. To establish whether the above results were due to damage of the spinal cord during enucleation, control experiments were carried out in four normal tadpoles by transecting the dorsal half of the spinal cord and adopting a 24-h survival time, as for grafted eyes. No terminal degeneration was found in the solitary tract (Fig. 3*b*).

To understand why ectopic fibres did not reach the optic tectum (the main natural target of optic fibres¹⁹), we removed both normal eyes of the host embryos at stage 26, that is, about

Fig. 4 *a*, Same experimental situation as Fig. 2, except that both normal optic vesicles of the host embryo were removed 12 h after eye transplantation. Horizontal section; rostral is left. Terminal degeneration is in the posterior part of the right optic tectum (the phenomenon was bilateral). During larval stages the posterior optic tectum is not yet laminated. On the extreme right is the postero-lateral corner of the tectum and its border (arrow) with the cerebellum. In grafted and blinded tadpoles the terminal degeneration in the tectum was always accompanied by terminal degeneration in the solitary tract (see Fig. 3*a*) and by degenerating fibres in the spinal cord (see Fig. 2). *b*, Corresponding region of the left optic tectum of a triocular tadpole whose grafted eye successfully innervated the spinal cord. No terminal degeneration is present. The specimen shown here is the same one whose solitary tract is represented in Fig. 3*a*. Scale bar, 100 μ m.



pathways¹². The adoption of a certain route of growth and the choice of the direction of growth do not necessarily depend on the same mechanism. Are directional cues encoded in the overall polarity of the brain or in the location of the target relative to the graft? For optic fibres growing rostrally in the spinal cord, the latter would seem to be the case. The mechanism by which a target organ could guide axonal growth remains to be explained.

The plasticity of growing optic fibres is proved not only by their ability to follow unusual pathways in the CNS, but also by their anomalous termination. We found that rostrally growing ectopic fibres terminate in the nucleus of the solitary tract. Future ultrastructural and electrophysiological investigation will show whether functional synapses are formed within this nucleus, which normally receives visceral signals. Ectopic fibres were found to terminate in the caudal part of the optic tectum only in blinded animals. It is difficult to explain the lack of such projection in triocular animals in terms of competition for terminal space; at very young larval stages (when ectopic fibres could have reached the tectum) normal optic fibres terminate in the rostral part of the tectum, which becomes fully colonised only after stage 57 (refs 20, 21).

It will be necessary to analyse in detail the embryological nervous system as it interacts with growing ectopic axons, before attempting to understand the mechanisms underlying such interaction. In our view the adoption by the ectopic axons of a certain route may depend on the options offered by the experiment (for example, level of penetration into the CNS), and on a hierarchy of preferred interactions with the surrounding tissues (through cell surface recognition²²). The present work focuses attention on the role of a target, in this case the optic tectum; both its position (relative to the graft) and its input (from normal eyes) seem to affect the behaviour of optic fibres issuing from the grafted eye.

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Microscopic epididymides in testicular feminisation

MICE bearing the X-linked testicular feminisation (*Tfm*)¹ gene in the hemizygous (X/Y) state have been presumed to have no traces of the male genital system. Unlike normal genetic males², they are apparently unresponsive to the androgens produced by the developing testes^{3,4}, and it has been presumed that derivatives of the embryonic mesonephric (Wolffian) duct system (MDS) are unable to survive in *Tfm*/Y males. I describe here, however, microscopic components of the complex comprising rete testis, efferent ductules, epididymis and, sometimes, vas deferens in adult *Tfm*/Y mice. These structures are unrelated to the effects of the presumed mutation *O^h*, to which the microscopic epididymides of some *Tfm*/Y mice have been ascribed⁵.

Because it has not been known whether *Tfm*/Y mice without *O^h* possess microscopic epididymides, I examined such adults (6–33 weeks). As the *O^h* X chromosome carries the blotchy (*Blo*) mutation, mice with *Blo* (*n* = 3) and without (*n* = 2) were studied. They were bred from stock obtained from U. Drews, Münster, and J. Tønnes Nielsen, Aarhus, respectively. Both strains originate from Harwell.

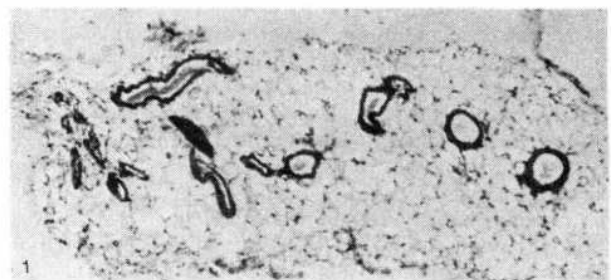
Right and left gonads, with paragonadal material from the caudal pole of the kidney to the internal inguinal ring, were fixed and embedded *en bloc* in paraplast. Serial 7–12 µm sections of the entire block (~1,500 sections per block) were stained with haematoxylin and eosin, and examined by light microscopy.

All the mice possessed rete testis, efferent ductules and microscopic epididymides on both sides (Fig. 1), and in each case there was continuity between epididymis and rete. The epididymides resembled those reported in *Tfm O^h Blo*/Y mice⁵. A vas deferens could sometimes be identified. The epithelium lining these mesonephric derivatives was mainly of the low cuboidal type attributed to *Tfm* cells^{5–8} in *Tfm*/+ heterozygotes masculinised by the sex-reversal gene *Sxr*⁹. However, isolated areas of taller, stereociliated or ciliated epithelium were seen (Fig. 2). No simple correlation between age and the degree of development of MDS was evident.

Microscopic epididymides are thus evidently a trait of *Tfm*, not of the *O^h* state. My observations also have a bearing on previous conclusions drawn from data on masculinised *Tfm*/+ heterozygotes. The assumption that the cuboidal epithelial cells in these heterozygotes represent surviving *Tfm* cells receives some support. There is, however, no need to suggest intercellular diffusion or metabolic co-operation^{5–8}; these cells are clearly capable of independent survival. Furthermore, stereocilia or cilia can no longer be used to distinguish *Tfm* from wild type cells⁸ in such heterozygotes. Indeed, there is no reliable criterion for this purpose.

The survival of epididymal and related structures in adult *Tfm*/Y mice suggests that the cells involved are either not dependent on testosterone or they are not entirely insensitive to it. Because the enzyme β-glucuronidase is histochemically

Fig. 1 Cross-section of the epididymis of a 9-week-old *Tfm*/Y mouse without *Blo* (×60).



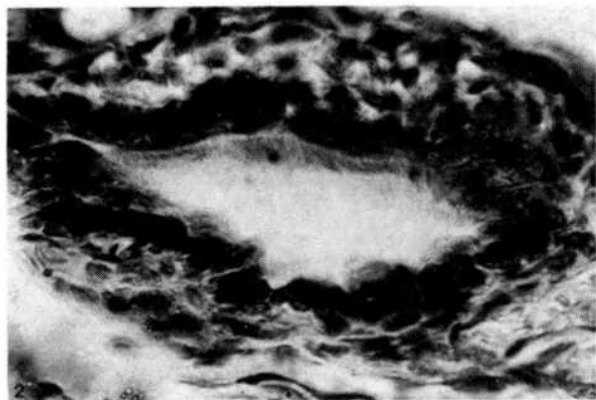


Fig. 2 Tubule cross-section of epididymis of a 9-week-old *Tfm/Y* mouse without *Blo* ($\times 750$), showing ciliated and/or stereociliated epithelium. (Both cilia and stereocilia appear to be present in normal mouse epididymides¹³.)

inducible by androgen in normal epididymis¹⁰, as in the kidney¹¹, studies of that enzyme in the microscopical epididymides may resolve this problem.

Not only the MDS but also the testis itself is abnormally small in *Tfm/Y* hemizygotes. It is possible that initial embryonic determination, establishment and maintenance of both testis and MDS are independent of testosterone but that normal maturation of both is under its control. Thus in one possible interpretation of my data, *Tfm* cells in these organs may be insensitive to androgens, but survive because only some aspects of normal function and development are dependent on such hormones. Alternatively, ontogenetic changes in affinity of repressor molecules for inducer (androgen), or for receptor sites, or developmental loss of the latter, could account for my observations.

Rete components of the MDS are essential for initiation of meiosis in the mouse testis¹², and in *Tfm/Y* mice meiosis is indeed initiated and proceeds to metaphase¹. On this basis the presence of a functional rete in *Tfm* mice could have been predicted. Conversely, it can be deduced from my observations that at least part of an MDS composed entirely of *Tfm* cells is functionally competent, independent of any metabolic co-operation with wild type cells. This system may be of value in studies of developmental aspects of hormonal competence.

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Uniparental inheritance is promoted by delayed division of the zygote in *Chlamydomonas*

SEXUAL REPRODUCTION in *Chlamydomonas reinhardtii*, a heterothallic, unicellular green alga, yields two types of zygotes: the thick-walled, dormant zygospore (meiotic), and less frequently, the vegetative zygote which divides mitotically soon after initial gamete fusion to produce stable diploid progeny^{1,2}. Although vegetative zygotes account for less than 5% of the products of gamete fusion, their diploid progeny can be easily

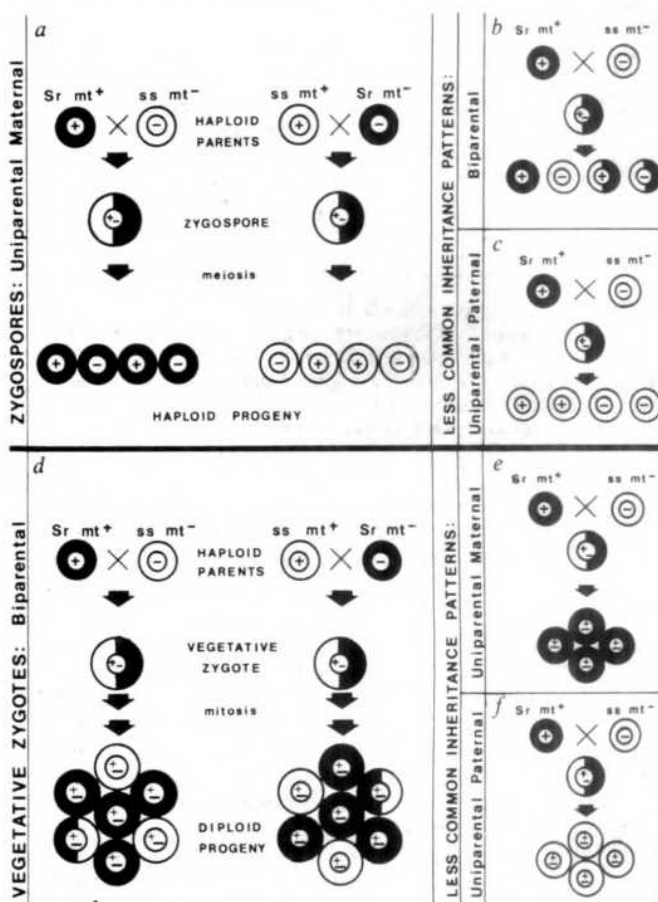


Fig. 1 Comparison of chloroplast gene transmission patterns in zygospores and vegetative zygotes of *C. reinhardtii*. Chloroplast gene transmission in zygospores: *a*, the chloroplast streptomycin-resistance mutation, *Sr* (shaded circles) is transmitted to all four meiotic products by greater than 90% of the zygospores if introduced through the mt⁺ parent, and is transmitted to none of the progeny if introduced through the mt⁻ parent. *b*, Biparental inheritance, observed for less than 10% of the zygospores, results in the transmission of both the *Sr* and wild-type *ss* (open circles) chloroplast alleles to the meiotic products. *c*, Paternal zygospores, transmitting only the chloroplast alleles of the mt⁺ parent, represent less than 1% of the zygospores in most crosses. Regardless of the pattern of chloroplast gene transmission, the nuclear mating-type alleles (indicated by + and -) segregate 2:2 among the meiotic products. Chloroplast gene transmission in vegetative zygotes: *d*, Biparental vegetative zygotes, transmitting the chloroplast alleles of both parents to their diploid progeny account for greater than 70% of the vegetative zygote population in most crosses. Segregation of parental chloroplast alleles occurs gradually and is completed within 20 generations. *e-f*, less than 25% of the vegetative zygotes exhibit uniparental chloroplast gene transmission, with uniparental maternal inheritance (*e*) usually being much more common than uniparental paternal (*f*). (The mendelian auxotrophic markers required for the selection and identification of vegetative zygotes² are not indicated in the figure.)

Table 1 Chloroplast gene inheritance patterns in zygospores and vegetative zygotes of *C. reinhardtii*

Cross	Chloroplast phenotypes	Chloroplast inheritance patterns (frequency)					
		Zygospores			Vegetative zygotes		
		BP	UPm	UPp	BP	UPm	UPp
<i>a</i> <i>Nr ss mt</i> ⁺ × <i>ns Sr mt</i> [−] <i>ns Sr mt</i> ⁺ × <i>Nr ss mt</i> [−]	Neamine-resistant						
	×						
	Streptomycin-resistant	0.02	0.97	0.01	0.90	0.08	0.02
	Streptomycin-resistant						
	×	0.01	0.97	0.02	0.94	0.06	0
	Neamine-resistant						
Chloroplast inheritance patterns (frequency)							
Vegetative zygotes							
		Isolated under continuous light			Isolated under continuous dark		
		BP	UPm	UPp	BP	UPm	UPp
<i>b</i> <i>Nr ss mt</i> ⁺ × <i>ns Sr mt</i> [−] <i>Er sps mt</i> ⁺ × <i>es Spr mt</i> [−] <i>es Spr mt</i> ⁺ × <i>Er sps mt</i> [−]	Neamine-resistant						
	×						
	Streptomycin-resistant	0.86	0.14	0	0.32	0.63	0.05
	Erythromycin-resistant						
	×	0.95	0.02	0.03	0.57	0.25	0.19
	Spectinomycin-resistant						
×							
Spectinomycin-resistant							
×							
Erythromycin-resistant	0.66	0.34	0	0.18	0.68	0.14	

Opposite mating types in all of the above crosses also carried recessive complementary mendelian mutations conferring a requirement for exogenous arginine. Thus, vegetative zygotes were selected by plating mated cells on medium lacking arginine (acetate was provided as an organic carbon source for all isolations). Zygospores were matured in darkness for 7–10 d on the same medium; germination was subsequently induced by transfer to medium containing arginine and incubation in the light. 50–64 vegetative zygotes and/or 100–200 zygospores were analysed for transmission of the chloroplast antibiotic resistance markers. Chloroplast inheritance patterns are abbreviated as follows: *BP*, biparental; *UPm*, uniparental maternal; *UPp*, uniparental paternal.

selected on minimal medium by crossing haploid strains which carry complementing auxotrophic mendelian mutations². I report here that differences in chloroplast inheritance observed between meiotic and mitotic zygotes of *Chlamydomonas* are correlated to the timing, rather than the nature, of their first divisions. Environmental conditions which delay the first post-mating division of vegetative zygotes result in uniparental inheritance while favourable post-mating environments promote biparental inheritance of chloroplast genes.

Chloroplast inheritance in *C. reinhardtii* is generally described as uniparental since 90% or more of the zygospores transmit to their progeny only those chloroplast alleles derived from the 'maternal' (mating-type plus, *mt*⁺) parent^{3–5}. Exceptional zygospores may transmit the chloroplast alleles of both parents (biparental zygospores), or rarely, may transmit only those alleles initially carried by the 'paternal' (mating-type minus, *mt*[−]) parent (Table 1*a*, Fig. 1). Nearly all investigations on chloroplast inheritance in *Chlamydomonas* have centred on the meiotic zygospore. The notable exceptions are studies by Gillham^{6,7} reporting a tendency toward biparental chloroplast gene transmission in vegetative zygotes (see Fig. 1) and suggesting a correlation between their inability to undergo meiosis and their failure to exclude paternal chloroplast alleles.

In work to be reported elsewhere, I found that the culture medium used to isolate vegetative zygotes affected the pattern of chloroplast gene transmission. Media promoting the most rapid vegetative growth also promoted biparental inheritance. This suggested that the observed differences in inheritance patterns between zygospores and vegetative zygotes (see Table 1*a*, Fig. 1) might result from differences in the timing, rather than in the nature (meiotic or mitotic), of their first post-mating divisions. To test this hypothesis I attempted to induce a temporary dormancy in the vegetative zygotes. Experimental procedures which can be used to promote maturation and extend dormancy in zygospores include nitrogen starvation⁸ and incubation in darkness⁹; it seemed likely that either of these treatments might also delay the first division of vegetative zygotes.

When the vegetative zygotes were selected in darkness, the recovery of macroscopically visible zygote clones was delayed as

compared to control isolations under continuous illumination. Furthermore, chloroplast gene transmission shifted toward uniparental maternal inheritance (Table 1*b*). By returning the vegetative zygotes to the light after various periods in darkness, a stepwise change in the inheritance pattern can be observed (Fig. 2*a*). However, as vegetative zygotes (but not zygospores) do eventually divide in darkness (if an organic carbon source is provided), this method does not make it possible to delay the first division indefinitely.

In contrast, the nitrogen-free medium used to induce gametogenesis and mating in *C. reinhardtii*¹⁰ also blocks post-mating cell division. By continuing to starve cells for nitrogen after mating, division of the vegetative zygotes can be delayed for at least 6 d with no subsequent loss in viability. Increasing the duration of the post-mating nitrogen starvation period led to a decrease in the frequency of biparental chloroplast gene transmission until uniparental maternal inheritance, normally associated with zygospores, became the predominant inheritance pattern in the vegetative zygote population also (Fig. 2*b*).

To verify that the experimental conditions of dark incubation or nitrogen starvation delayed the first division of the vegetative zygotes, and to estimate the approximate extent of this delay, subsamples of the mating population were plated on standard agar-solidified medium and incubated in various conditions. Periodically, sterile liquid was added to the surface of the sample plates and the adherent cells were respread. Respreading after zygote division had occurred would be expected to result in an increase in the number of colonies subsequently recovered.

In this manner, it was shown that: (1) incubation in darkness delayed the onset of the first division by approximately 40 h (Fig. 3*a*); (2) no division of the vegetative zygotes occurred during maintenance in nitrogen-free medium (Fig. 3*b*), and (3) under continuous illumination, division always occurred within 30 h after the return of available nitrogen, regardless of the duration of the previous dormant (nitrogen-starvation) period.

While these observations demonstrate a previously unrecognised potential for uniparental inheritance in vegetative zygotes, they perhaps more importantly reveal a remarkable influence of external environment on chloroplast inheritance. This influence,

which extends also to zygospores of *C. reinhardtii*^{8,11,12}, must be borne in mind when defining laboratory conditions for studies on chloroplast inheritance, and when interpreting and comparing the results of such experiments conducted in different laboratories.

The correlation between delayed division and uniparental inheritance suggests an alternative to previous arguments that uniparental inheritance is, in itself, of direct selective advantage^{13,14}. The molecular mechanism responsible for uniparental inheritance in *Chlamydomonas* apparently involves extensive degradation of zygotic chloroplast DNA (normally present as multiple copies of a small genome in vegetative cells)¹⁵⁻¹⁸. Perhaps chloroplast genome polyploidy is a luxury permitted only in an optimum environment, and reduction in ploidy level represents an energy-conserving measure essential to the maintenance of viability in a hostile environment. Viewed in this way, uniparental inheritance is seen as an indirect consequence of direct selection, in evolutionary history, for the dormant, highly resistant stage (the zygospore) in the life cycle of *Chlamydomonas*.

Fig. 2 Effect of post-mating environmental conditions on the transmission of chloroplast genes in vegetative zygotes of *C. reinhardtii*. *a*, Effect of post-mating incubation in darkness. Chloroplast mutants resistant to neomycin (*mt*⁺ parent) and to streptomycin (*mt*⁻ parent) were crossed following standard procedures^{8,9} and the mated cells plated on acetate-supplemented minimal medium for the selection of vegetative zygotes (complementary recessive nuclear mutations to arginine auxotrophy were carried by opposite mating-types). Subsamples of the selection plates were incubated in darkness for the periods indicated and were then transferred to continuous light. Resultant zygote colonies were replica-plated to medium supplemented with 75 $\mu\text{g ml}^{-1}$ neomycin and to medium supplemented with 100 $\mu\text{g ml}^{-1}$ streptomycin. Biparental vegetative zygote clones are defined as those which yield growth on both of the antibiotic test media. *b*, Effect of post-mating nitrogen starvation. Parental strains are as described in (*a*) above. Gametogenesis and mating were carried out in nitrogen-free medium and the mated culture held in the same medium for 6 d. Samples were periodically withdrawn at the times indicated and plated on nitrogen-containing, acetate-supplemented standard medium under continuous illumination. Biparental zygote clones were defined as described above. Open and shaded circles represent separate repetitions of the same cross. 64 vegetative zygote clones were analysed from each dark incubation (*a*) or nitrogen-starvation (*b*) period. Vertical bars indicate 95% confidence limits.

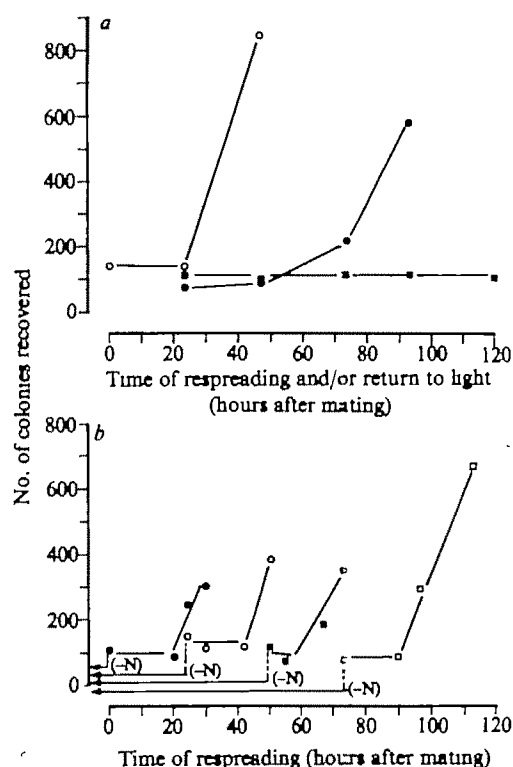
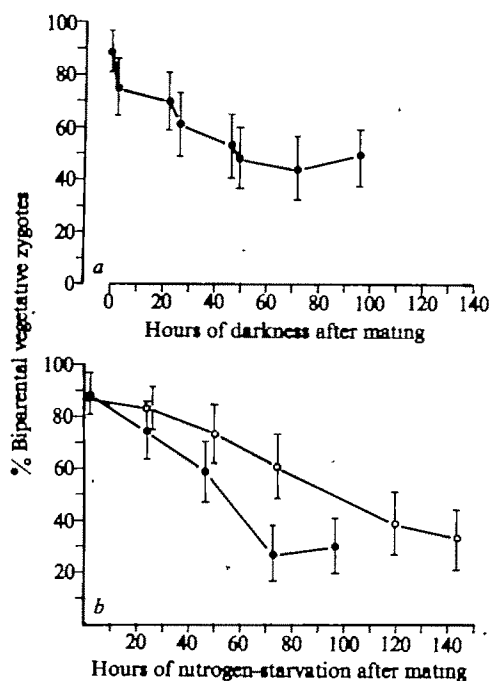


Fig. 3 Effect of post-mating environmental conditions on the timing of the first division of vegetative zygotes of *C. reinhardtii*. *a*, Effect of post-mating incubation in darkness. Mated cells were plated on acetate-supplemented minimal medium and incubated either under continuous illumination (○) or in darkness (●, ■). At the times indicated, the adherent cells were respread. Sample plates incubated in darkness were returned to the light at each indicated time both with (●) and without (■) respreading. The time of the first vegetative zygote division can be estimated by comparing the number of colonies recovered on respread and non-respread plates. *b*, Effect of post-mating nitrogen starvation. Mated cells were removed from nitrogen-free medium and were plated on acetate-supplemented minimal medium within 2 h after mating (●), and after 24 (○), 48 (■), or 72 (□) h of post-mating nitrogen starvation. Adherent cells were respread periodically after plating (the first symbol, in each case, indicates the average colony count on non-respread plates).

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Sensitivity of corticosteroid-dependent insulin-resistant lipogenesis in marrow preadipocytes of obese-diabetic (db/db) mice

It is well known that adipocytes with specific cell-membrane insulin receptors respond to insulin by increasing glucose uptake, oxidation and lipogenesis in the presence of glucose¹⁻⁴. Maintenance of adipocyte insulin sensitivity during a state of relative muscle cell insulin resistance has been considered a possible mechanism for obesity in diabetes mellitus⁵. I now report that a distinct cell phenotype resists insulin but accumulates lipid in response to corticosteroid. Furthermore, corticosteroid-induced lipogenesis is significantly greater in marrow preadipocytes of mutant diabetic-obese mice.

Weanling 6-8 week-old NIH/Swiss, BALB/c (National Cancer Institute), C57 BL/KsJ-db (genotype *db+/db+*) diabetic mice and their control littermates *db+/m+* and *m+/m+* (Jackson Laboratories) were killed by cervical dislocation and the marrow contents of a single femur and tibia were flushed in 10.0-ml volumes of Fisher's medium (Gibco), supplemented with 25% serum (donor horse, fetal calf or goat), into plastic flasks (Corning) as before⁶ and standardised to 2.0×10^7 cells per culture. A sample of donor horse serum (Flow Laboratories, Manchester) which has been reported to induce lipid accumulation in marrow cells⁶, was provided by Dr T. M. Dexter. The serum factor(s) stimulating lipid accumulation by these cells and their role in maintaining haemopoiesis *in vitro* are unknown.

Fig. 1 Morphological appearance of the adherent cell population in a 4-week marrow culture grown in: *a*, 'deficient' lot 4107064 horse serum ($\times 100$); or *b*, the same serum supplemented with 10^{-7} M hydrocortisone sodium hemisuccinate ($\times 200$).

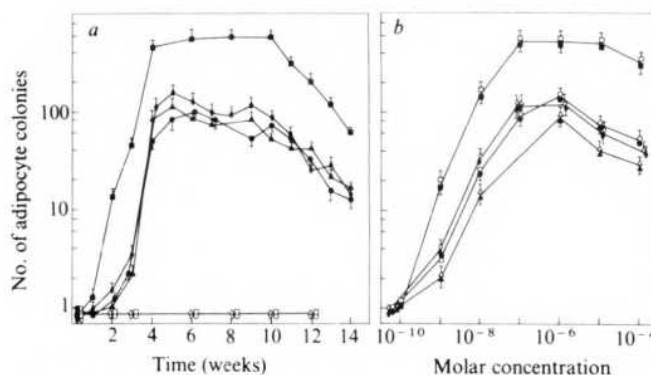
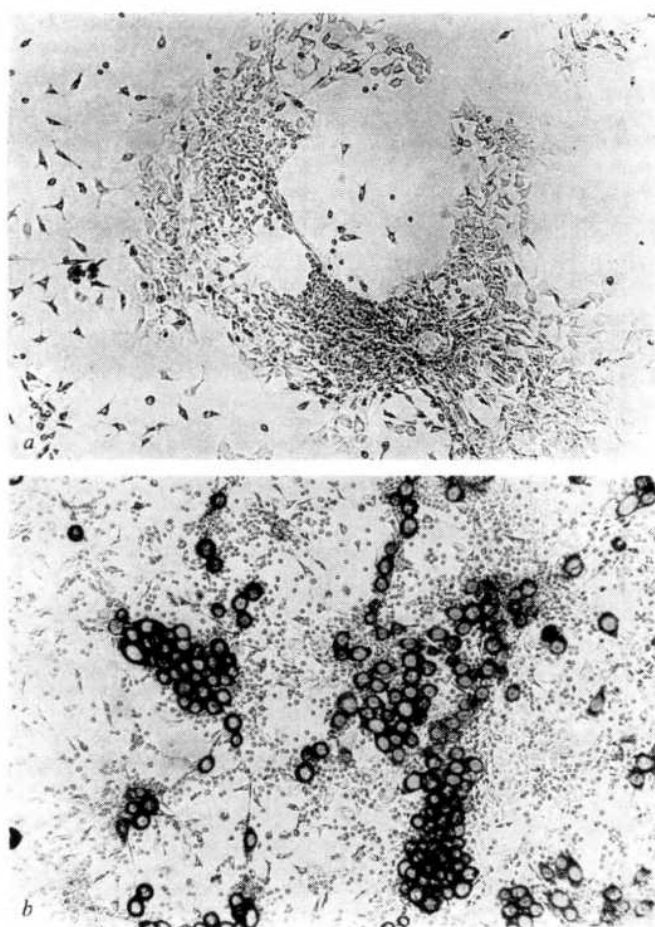


Fig. 2 Kinetics and sensitivity of corticosteroid-induced adipocyte colony formation in obese-diabetic compared with control mouse marrow. *a*, Results are the mean of at least three cultures at each time grown in 10^{-7} M insulin (open symbols) or 10^{-7} M hydrocortisone sodium hemisuccinate (closed symbols) for each mouse strain; *b*, results are the mean of total adipocyte colonies per flask scored at week 6 for at least three cultures at each concentration of hydrocortisone sodium hemisuccinate alone (open symbols) or in the presence of 10^{-7} M insulin in addition to steroid (closed symbols). $\square$, C57BL/Ks-J (db/db); $\circ$, C57BL/Ks-J (db/m or m/m); $\diamond$, NIH/Swiss; and $\triangle$, Balb/c.

Cultures were incubated at 33, 31 or 37 °C and were depopulated and fed by weekly removal of half the flask volume (5.0 ml) and replacement by an equal volume of fresh medium. Cultures were recharged at week 4 by addition of the contents of a second mouse femur⁶. Sera from several sources were supplemented with each of several concentrations of hormones listed in Table 1, and cultures were scored daily for the number of macroscopic adipose cell-containing colonies per flask and of adipose cells per high-powered microscopic field (Olympus inverted microscope). As Table 1 shows, Manchester serum supported formation of more than 100 macroscopic adipose cell colonies per flask by week 4. This was associated with more than 100 adipocytes per high-powered field in the centre of such a colony. Each of 16 lots of horse serum (Flow Laboratories, Rockville), eight lots of fetal calf serum (Colorado Serum Co. or Gibco), or goat serum in concentrations ranging from 10 to 50% failed to stimulate adipose cell differentiation (Table 1). Addition of arachidonic acid (Nu-chek, Elysian, 0.5–500 $\mu\text{g ml}^{-1}$), bovine insulin (Sigma, 0.5–500 $\mu\text{g ml}^{-1}$), butyric acid, dimethylsulphoxide or idoxuridine to deficient sera failed to stimulate adipocyte differentiation.

In contrast, addition of hydrocortisone sodium hemisuccinate (Solu-Cortef, Upjohn) in doses between 0.05 and 500 ng ml^{-1} (10^{-10} – 10^{-6} M) resulted in dose-dependent adipose cell differentiation (Fig. 1) with 12 of the 16 lots of donor horse serum and to a lesser extent with fetal calf or goat serum (Table 1 and Fig. 2). Addition of corticosteroid to Manchester serum did not further increase the numbers or size of adipocyte colonies. Removal of corticosteroid from the medium after adipocytes had differentiated was associated with gradual loss of adipocytes. Thus, the continued presence of corticosteroid was absolutely required.

Other corticosteroids were effective as well as the mineralocorticoid, *d*-aldosterone and the 17-hydroxy adrenal corticosteroid corticosterone (Table 1). Oestrogenic, androgenic or progestational steroids were inactive in the range 10^{-10} – 10^{-4} M (Table 1). With optimal corticosteroid concentration, 10^{-7} M, adipocyte differentiation increased directly with serum concentration in the range 0.5–25.0%, thus arguing against the presence of a serum inhibitor. Corticosteroid-induced adipocyte differentiation was observed in cultures at 31 and 37 °C but was optimal at 33 °C.

Marrow from C57BL/Ks-J (db/db), (db/m), (m/m), BALB/c and NIH/Swiss mice was next compared for steroid sensitivity of adipocyte differentiation. There was no detectable lipid accu-

mulation in *db+db+* or control littermate marrow preadipocytes in response to increased fatty acid concentration or insulin (Fig. 2a). When 2×10^7 marrow cells were cultured in 10^{-7} M hydrocortisone, *db/db* preadipocytes yielded significantly more adipocyte colonies after 8 weeks than did preadipocytes from control littermates. Figure 2b shows that this reflected a true increase in sensitivity of *db/db* cells to corticosteroid. Significant adipocyte differentiation was detected at a corticosteroid concentration (10^{-9} M) 100 times lower than that required by marrow from control littermates.

Evidence that marrow preadipocyte differentiation was truly insulin independent was provided as follows. Cultures were preincubated in insulin (10^{-10} – 10^{-7} M) for up to 4 weeks with no alteration in the number of adipocyte colonies induced after the addition of 10^{-7} M corticosteroid at week 4. Addition of insulin to corticosteroid-containing cultures of *db/db* or control marrow did not further stimulate or block lipogenesis (Fig. 2b).

When adipocytes differentiated in hydrocortisone-treated cultures 5–7 times as many adherent cells and 100–500 times as many nonadherent cells were detected in culture flasks at weeks 4, 8, 12 and 14 as in untreated cultures. There was persistent proliferation of pluripotent haemopoietic stem cells (CFUs) and GM-progenitor cells (CFUc) for at least 14 weeks in steroid-treated cultures while untreated cultures failed to support haemopoiesis after week 6 (Fig. 3). It is not known whether the increased numbers of CFUc and CFUs arose from corticosteroid-induced cells or were present in the inoculum and required corticosteroid for their proliferation or maintenance of a growth stimulatory microenvironment. Recent data indicate that when preadipocyte differentiation is blocked by Kirsten murine sarcoma virus, *in vitro* haemopoiesis¹⁶ is inhibited.

Epitidymal adipocytes resist lipogenesis by insulin after insulin-preincubation and this has been shown to represent

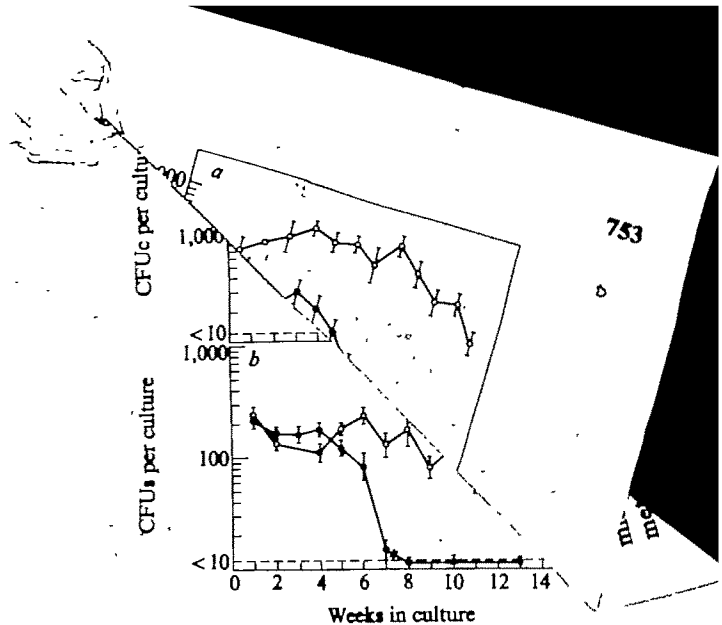


Fig. 3 Influence of corticosteroid-induced marrow preadipocyte differentiation on pluripotent haemopoietic stem cell and GM-progenitor cell proliferation *in vitro*. Cultures of NIH/Swiss mouse marrow were collected weekly by removal of half the flask volume (5.0 ml) and assayed for total numbers of spleen colonies (CFUs) and *in vitro* GM colonies (CFUc)⁶⁵ detected per 10^5 cells. Results are corrected for the total number of colony-forming cells in the 5.0-ml removed and are the mean and standard error for at least three flasks at each week. a, CFUc for 25% 'deficient' lot 4107064 horse serum alone, ●; or with 10^{-7} M hydrocortisone sodium hemisuccinate, ○. b, CFUs for 25% 'deficient' horse serum alone, ●; or with 10^{-7} M hydrocortisone sodium hemisuccinate, ○.

Table 1 Effect of steroids on *in vitro* mouse bone marrow preadipocyte differentiation

Serum with or without steroid (molar concentration)	No. adipose cell islands/culture					No. adipocytes/ high powered field				
	1	2	4	8	15	1	2	4	8	15
Fetal calf serum										
Colorado alone	0	0	0	0	0	8	3	1	0	0
Colorado+hydrocortisone										
Sodium hemisuccinate (10^{-7})	10	16	45	30	17	50	75	85	75	71
Gibco+hydrocortisone										
Sodium hemisuccinate (10^{-7})	8	35	18	21	16	13	57	70	65	60
Gibco alone	0	0	0	0	0	4	0	0	0	0
Goat serum alone	0	0	0	0	0	8	0	0	0	0
Goat serum+hydrocortisone										
Sodium hemisuccinate (10^{-7})	8	10	6	1	1	4	18	25	25	18
Horse serum										
Flow Laboratories (Manchester)	33	40	>100	>100	53	79	>100	>100	>100	50
Flow Laboratories (Rockville)										
Lot 4107064	0	0	0	3	0	8	1	10	0	0
Lot 29211001	0	0	0	0	0	0	0	0	0	0
Lot 29210011	0	0	0	0	0	0	0	0	0	0
Lot 4017064+hydrocortisone										
Sodium hemisuccinate (10^{-7})	41	>100	>100	>100	41	>100	>100	>100	>100	31
+Methylprednisolone sodium hemisuccinate	(10^{-7})	50	>100	>100	61	>100	>100	>100	>100	41
+Cortisone acetate	(10^{-7})	51	>100	>100	13	>100	>100	>100	>100	50
+Dexamethasone phosphate	(10^{-7})	10	>100	>100	61	>100	>100	>100	>100	40
+Prednisolone sodium phosphate	(10^{-7})	8	>100	41	38	22	17	51	>100	30
+Testosterone enanthate	(10^{-7})	0	0	0	0	0	6	0	0	0
+Oestradiol valerate	(10^{-7})	0	0	0	0	0	8	0	0	0
+ Δ -Aldosterone	(10^{-7})	10	30	43	>100	30	6	>10	>10	40
+Testosterone	(10^{-7})	0	0	0	0	0	10	0	0	0
+Androstenedione	(10^{-7})	0	0	0	0	0	5	0	0	0
+Corticosterone	(10^{-7})	0	20	30	>100	20	8	40	>100	30
+Dehydroepiandrosterone sulphate	(10^{-7})	0	0	0	0	0	10	0	0	0
+ β -Oestradiol	(10^{-7})	0	0	0	0	0	6	0	0	0
+Oestrinol	(10^{-7})	0	0	0	0	0	5	0	0	0
+Oestrone	(10^{-7})	0	0	0	0	0	8	0	0	0
+Progesterone	(10^{-7})	0	0	0	0	0	10	0	0	0
+Pregnenolone	(10^{-7})	0	0	0	0	0	9	0	0	0

Each week drugs (Sigma) were dissolved in ethanol or acetone according to published procedures in 1,000-fold concentrations and added in 0.01-ml volumes to each 10-ml culture flask. Results are the mean for at least three cultures in each set of conditions. At least 10 microscopic fields were scored for each culture. The cortisol concentration measured by radioimmunoassay in Manchester horse serum ($10.0 \mu\text{g ml}^{-1}$) did not differ significantly from that found in six lots of Rockville horse, fetal calf or goat serum (range 2.5–25 $\mu\text{g ml}^{-1}$). No difference was detected in corticosterone or Δ -aldosterone concentrations. These levels reflect total protein-bound and free corticosteroid. The biologically active unbound fraction was increased variably by addition of free steroid⁽¹⁵⁾. Results are for NIH/Swiss mouse marrow.

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block of insulin receptors³. Furthermore, β -galactosidase (G_{M1}) deficiency in non-marrow reported preincubation block of teroids directly or indirectly in marrow preadipocytes accelerate lipolysis of triglycerides in marrow. It will be here^{7,8}. Thus, the same metabolic pathways as the insulin-corticosteroid adipocyte and the 3T3-L1 cell line^{3,4,9}. Prof marrow preadipocytes in mutant obese-diabetic indicates that the gene for obesity¹⁰⁻¹⁴ affects lipid metabolism in multiple cell phenotypes.

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Enzyme replacement using liposome carriers in feline G_{M1} gangliosidosis fibroblasts

CURRENT emphasis in research on lysosomal storage diseases is directed towards enzyme replacement therapy (ERT) for these inherited enzyme deficiencies¹⁻³. The rationale for ERT is based on the assumption that endocytosed enzyme would enter lysosomes and function in the degradation of accumulated substrates. However, inactivation of enzyme, immunological reactions and low cellular uptake have prevented successful application of this promising approach to therapy. Circumvention of these limitations requires the use of carriers which protect and stabilise replacement enzyme while enhancing uptake and subcellular distribution²⁻⁵. We report here the use of lipid vesicles (liposomes)⁶⁻⁹ as carriers for replacement enzyme in feline G_{M1} gangliosidosis fibroblasts which have deficient β -galactosidase (β -gal) activity and lysosomal storage of glycopeptides⁹⁻¹¹. Our results demonstrate increased β -gal activity and corresponding catabolism of stored glycopeptides in feline G_{M1} gangliosidosis fibroblasts treated with liposomes containing β -gal.

Fibroblast cultures derived from skin of normal cats and cats with G_{M1} gangliosidosis were maintained in McCoy's 5a medium with 20% fetal calf serum¹². β -Galactosidase activity was determined with the fluorogenic substrate, 4-methyl-umbelliferyl- β -D-galactoside (4-MU- β -galactoside) (1 unit = 1 nmol substrate cleaved h⁻¹)^{10,13}, and protein was estimated by the

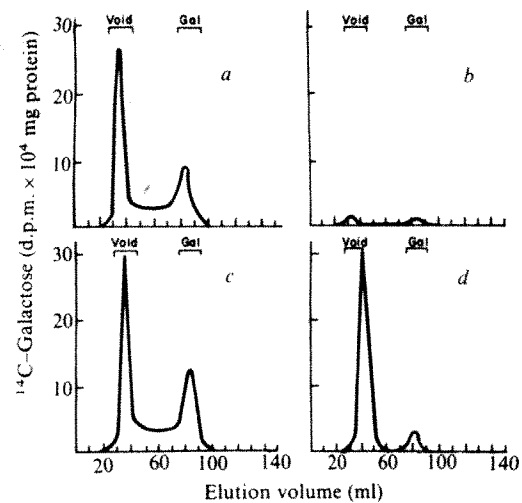


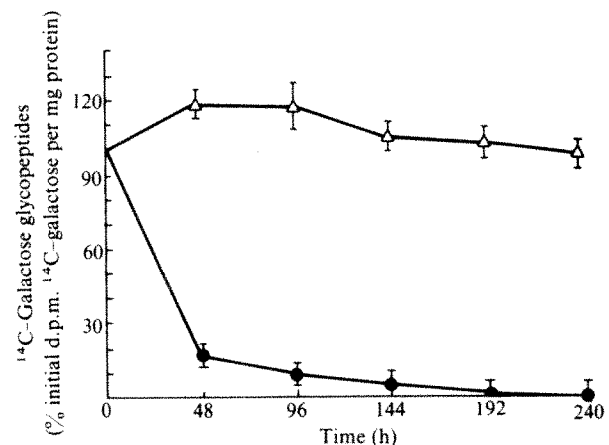
Fig. 1 Elution patterns of ¹⁴C-GP from a 0.9 × 100 cm BioGel P10 column. Homozygous dominant fibroblasts at: a, end of pulse and b, 240 h after pulse, and homozygous recessive fibroblasts at: c, end of pulse and d, 240 h after pulse. (4 ml fractions.)

method of Lowry¹⁴. Partially purified β -gal was obtained by concanavalin A chromatography of acetone-precipitated normal feline liver homogenate¹⁰. Large multilamellar liposomes (1,000-6,000 Å) containing β -gal [L(β -gal)] or thorium dioxide (L[thor]) were prepared from phosphatidylcholine, dicetyl phosphate and cholesterol in a molar ratio of 7:2:1 (refs 5-8). Emulsification of the lipids in 6 ml of aqueous β -gal or thorium dioxide in a bath-type sonicator produced liposomes containing 11-13% of the total aqueous solute.

Entrapment of solutes within aqueous interstices of liposomes was confirmed by chromatographic separation of liposomes from untrapped solute and by demonstration of latency of entrapped solutes⁸. The integrity of the liposomes during incubation with fibroblasts was demonstrated by similar analysis of spent culture fluid⁸. In all cases, there was retention of entrapped solutes within chromatographic fractions containing liposomes. Entrapped β -gal was shown to be latent until the liposomes were lysed with Trixon X-100.

Normal and G_{M1} gangliosidosis fibroblasts were incubated with 2 μ Ci ¹⁴C-galactose to label the endogenous glycopeptide pool^{11,15}. The degree of labelling and metabolic fate of ¹⁴C-galactose-labelled glycopeptides (¹⁴C-GP) were evaluated at 48-h intervals by chromatographic separation on BioGel P10. The elution patterns of ¹⁴C-GP from normal and G_{M1}

Fig. 2 Dynamics of ¹⁴C-GP metabolism in (●) homozygous dominant and (△) homozygous recessive fibroblasts following a 48-h pulse with 2 μ Ci ¹⁴C-galactose per mg protein.



gangliosidosis fibroblasts are shown in Fig. 1. Total ^{14}C -GP was expressed for each interval as d.p.m. ^{14}C per mg protein in fractions 5–11. In normal fibroblasts there was initial labelling of ^{14}C -GP followed by rapid catabolism and clearance of ^{14}C , reflecting normal β -gal activity. However, in G_{M1} gangliosidosis cells β -gal deficiency was expressed by retention of ^{14}C -GP (Fig. 2).

Uptake and intracellular fate of liposomes were evaluated by electron microscopy of fibroblasts which had been incubated with L[thor]. Monolayers were incubated with 2,000 μg liposomal lipid for 6 h, washed three times, detached with 0.25% trypsin, centrifuged and prepared for electron microscopy¹⁶. The intracellular accumulation of thorium dioxide within heterophagosomes and membranous residual bodies is shown in Fig. 3.

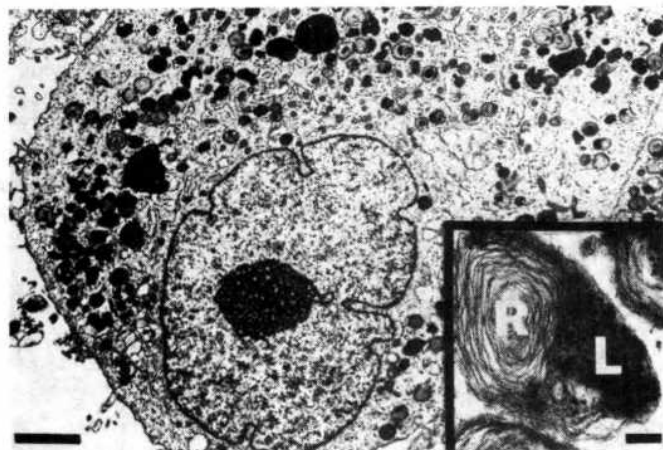


Fig. 3 Electron micrograph of a G_{M1} gangliosidosis fibroblast following incubation with L[thor] demonstrating the presence of thorium dioxide within the lysosomal apparatus of the cell (bar = 1.0 μm). Insert illustrates fusion between a secondary lysosome (L) containing Thorotrast and a membranous residual body (R) characteristic of G_{M1} gangliosidosis fibroblasts (bar = 0.1 μm).

The efficacy of ERT was evaluated by labelling G_{M1} gangliosidosis fibroblasts with ^{14}C -galactose as described above. After 48 h, each monolayer was treated with either L[β -gal] containing 3,000 units of β -gal activity and 2,000 μg liposomal lipid or 3,000 units of untrapped β -gal. Control samples consisted of untreated fibroblasts, fibroblasts treated with liposomes containing buffer, or fibroblasts treated with liposomes containing buffer plus untrapped β -gal (3,000 units). Treatments were repeated and samples collected in 48-h intervals. Changes in ^{14}C -GP were monitored as described above, and changes in enzyme activity were monitored by assay of cell lysates. Treatment with either unencapsulated β -gal or any of the control treatments had no effect on ^{14}C -GP storage. However, treatment with L[β -gal] resulted in clearance of >80% of ^{14}C -GP and increased β -gal activity to 70% of normal, reflecting uptake of approximately 10% of the total enzyme activity presented in liposomes (Fig. 4).

Characterisation of this *in vitro* system, in which the dynamics of naturally occurring lysosomal storage can be quantitated, provides a unique model for precise assessment of ERT. Endogenous storage material in feline G_{M1} gangliosidosis fibroblasts seems to be similar to that found in human G_{M1} gangliosidosis fibroblasts¹¹. This glycopeptide is structurally similar to storage material found in hepatocytes of human and feline G_{M1} gangliosidosis^{17,18}, suggesting a potentially meaningful analogy between *in vitro* and *in vivo* therapeutic trials.

The ultrastructure of cells treated with L[thor] confirmed the lysosomotropic effect of liposomes. Although fusion of liposomes with primary lysosomes has been documented previously⁸, this study clearly demonstrates fusion of liposomes with membranous residual bodies characteristic of many

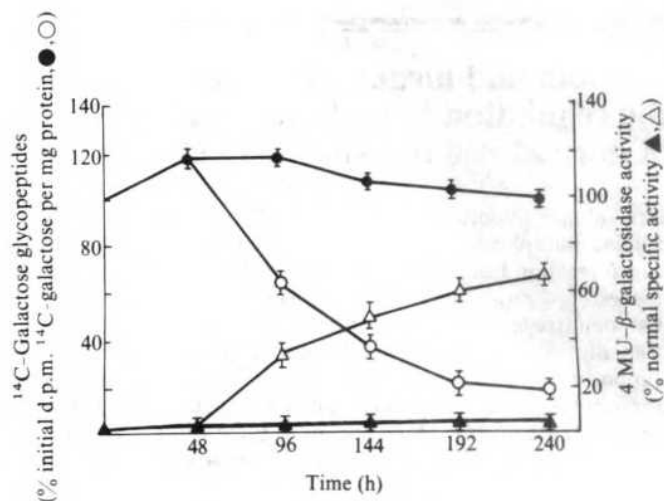


Fig. 4 Effect of enzyme replacement therapy on ^{14}C -GP storage and β -gal activity of G_{M1} gangliosidosis fibroblasts treated with: ●, unencapsulated β -gal, or ○, △, L[β -gal]. Differences in ^{14}C -GP storage and β -gal activity of all control samples and samples treated with unencapsulated β -gal were not statistically significant.

lysosomal storage diseases. This observation is important evidence supporting the potential value of liposome carriers in the prevention and reversal of lysosomal storage.

Failure of fibroblasts treated with unencapsulated enzyme to increase enzyme activity or degrade accumulated substrate supports the contention that a carrier is needed to introduce therapeutic quantities of hydrolytically active enzymes. Furthermore, increased levels of appropriate enzyme activity and enhanced degradation of endogenously accumulated substrate clearly demonstrate the value of liposomes as carriers of replacement enzyme in the correction of a metabolic defect in fibroblasts.

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Calcium and magnesium ions and the regulation of multiplication in normal and transformed cells

THE relative importance of Ca^{2+} and Mg^{2+} in the regulation of cellular multiplication and alteration of that regulation by viral transformation has recently become a subject of considerable interest¹⁻⁵. Extracellular Ca^{2+} and Mg^{2+} both have effects on metabolic parameters and macromolecular synthesis in cultured cells. Mg^{2+} , because of its substrate level intracellular concentration and its role in macromolecular synthesis and intracellular transphosphorylation reactions, has been suggested as a possible coordinate regulator of the positive pleiotropic response, a coordinated array of reactions associated with initiation of DNA synthesis and multiplication in cultured cells⁶⁻⁹. Ca^{2+} , on the other hand, has been suggested as a short term regulator of cell growth and function, largely on the basis of its tightly regulated low cytoplasmic concentration^{10,11}. In this report, we describe a kinetic analysis of the effects of extracellular concentrations of Ca^{2+} and Mg^{2+} on survival and multiplication of normal and transformed human cells in culture. We have found that cellular

multiplication rate (number of population doublings per unit time) and also cellular 'survival rate' (percentage survival divided by time) can be substituted in place of reaction rate in classical enzyme kinetic formulae (Fig. 1). Both responses exhibit saturation kinetics, with a transition from first order response to zero order, as the concentration of calcium, magnesium, or any other essential component of the culture medium is increased. Rate plotted against concentration yields the expected right rectangular hyperbola described by the Henri-Michaelis-Menten formulation, and the expected linear relation when plotted by the Lineweaver-Burke or van Hofstee methods. Linear regression of the Lineweaver-Burke double reciprocal plot yields from its intercepts a maximal rate for multiplication or survival (equivalent to V_{max} in classical enzyme kinetics) and a Michaelis-Menten constant (K_m), equal to the substrate (or ligand) concentration yielding a half maximal rate of multiplication or survival.

In order to apply Michaelis-Menten kinetic analyses to cellular multiplication and survival, we have made the following assumptions: (1) each substrate or ligand (S) interacts with sites on or in each cell (C) to form complexes (CS), operationally equivalent to the ES complex of first order enzymatic reactions; (2) although each cell may have many sites that form complexes with S, the interactions between these sites and S are such that

Fig. 1 Multiplication response of normal and transformed human lung fibroblasts to Mg^{2+} . *a*, Colony size: previously described procedures for clonal growth and measurement of colony size¹²⁻¹⁴ were used, with the modifications described below. The basal medium was MCDB 105 (ref. 14) with pantothenate supplied as a sodium salt (Sigma), and with calcium chloride and magnesium sulphate omitted. For these experiments the basal medium was supplemented with 1.0×10^{-3} M ultrapure grade $\text{CaCl}_2 \cdot 4\text{H}_2\text{O}$ (Alfa) and $250 \mu\text{g ml}^{-1}$ dialysed fetal bovine serum protein (FBSP)¹⁵. Ultrapure grade $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (Alfa) was added as indicated. Polylysine-coated tissue culture Petri dishes were used for N-HLF¹⁶. Uncoated dishes were used for SV-HLF, whose multiplication is either unaffected or slightly inhibited by the basic polymer. Each 60-mm dish was inoculated with 100 cells and incubated for 14 d. ●, Normal cell data for MRC-5 (ATCC CCL 171) at passage 20. Similar results have also been obtained with WI-38 and FLOW-2000 cells. ○, Transformed cell data for SV40 virus transformed WI-38 (Subline 2RA, ATCC CCL 75 1). The size of the five largest colonies per plate was measured photometrically with an aperture of 7 mm for N-HLF and 4 mm for SV-HLF as previously described¹². We have shown experimentally by trypsinisation and direct cellular counting with a Coulter counter that the photometric measurement averaged over the five largest colonies per dish is linearly related to the total number of cells per Petri dish (the sum of all colonies) and to the number of cells per colony in the colonies that are measured. Thus, the photometric measure can be used as an accurate indicator of total multiplication during the incubation period. *b*, multiplication rate: During the observation periods used here (7–14 d), the increase in colony size is essentially exponential. The increase in $\log C$ against t , where C is colony size and t incubation period, measured either by photometric or direct counting methods has a linear correlation coefficient $> +0.93$ for any given set of experimental conditions during that time period. Experimental data were normally collected after 14 d of incubation to minimise error in measurement of very small colonies. Colony size in photocell units was converted to multiplication rate by the formula $r = 3.32(\log_{10} C_t - \log_{10} C_0)/t$ where r = cell generations (population doublings) per d, t = incubation period in d, 3.32 = reciprocal $\log_{10} 2$, C_t = colony size in photocell units at the end of t d, and C_0 = colony size in photocell units at time zero. C_0 is a general background constant determined by linear regression to $t = 0$ of a plot of $\log_{10} C$ against t . For N-HLF, $C_0 = 0.080$ photocell units, and for SV-HLF, $C_0 = 0.11$ photocell units. The experimentally determined multiplication rates (r) for the various concentrations of Mg^{2+} have been fitted to a right rectangular hyperbola according to the Henri-Michaelis-Menten formulation. *c*, Lineweaver-Burke double reciprocal plot of the data from *b*. Values for the Michaelis-Menten constant (K_m) corresponding to the concentration of Mg^{2+} yielding a half maximal multiplication rate, and for r_{max} are obtained from the intercepts as shown. The best linear fit to the data was determined by 'least squares' estimate. Experiments resulting in regression lines with linear correlation coefficient $< +0.80$ were rejected for use in these analyses. Errors in K_m and r_{max} were estimated by Gaussian analysis of variance based on single data points which were means of triplicate determinations. Standard errors between triplicate individual determinations are indicated in *a* and *b* for N-HLF. For N-HLF, $K_m = 4.8(\pm 1.6) \times 10^{-5}$ M, $r_{\text{max}} = 0.492 \pm 0.055$, and for SV-HLF, $K_m = 4.2(\pm 1.0) \times 10^{-6}$ M, $r_{\text{max}} = 0.425 \pm 0.010$ at $P = 0.05$. The transformation of data on colony size and incubation time to a rate function distinguishes this analysis from previous studies seeking to apply enzyme reaction rate kinetics to cellular growth response, in which the fraction of cells synthesising DNA was measured rather than actual cellular multiplication rates¹⁷.

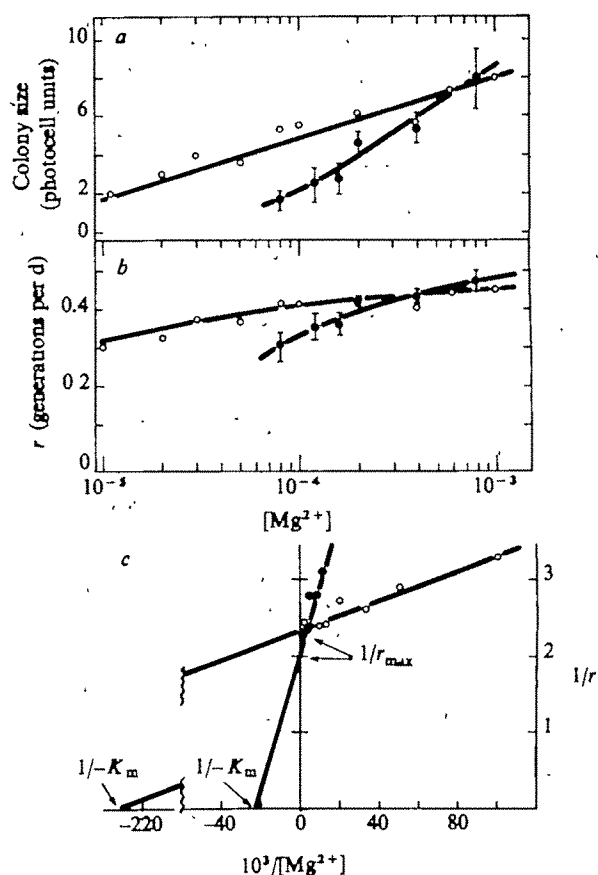


Table 1 Interaction of Mg^{2+} and Ca^{2+} in multiplication of normal and transformed human lung fibroblasts

Mg^{2+} (M)	N-HLF		SV-HLF	
	$K_m^{Ca^{2+}}$ ($mol\ l^{-1}$)	r_{max} (cell generations per d)	$K_m^{Ca^{2+}}$ ($mol\ l^{-1}$)	r_{max} (cell generations per d)
10^{-3}	$1.2(\pm 0.70) \times 10^{-5}$ NS	0.433 ± 0.025 ($P < 0.001$)	$1.1(\pm 0.64) \times 10^{-5}$ ($0.05 < P < 0.10$)	0.412 ± 0.025 ($0.02 < P < 0.05$)
10^{-4}	$1.5(\pm 1.25) \times 10^{-5}$	0.314 ± 0.039	$1.7(\pm 1.4) \times 10^{-5}$	0.398 ± 0.022
Ca^{2+} (M)	$K_m^{Mg^{2+}}$		$K_m^{Mg^{2+}}$	
	$K_m^{Mg^{2+}}$ ($mol\ l^{-1}$)	r_{max}	$K_m^{Mg^{2+}}$ ($mol\ l^{-1}$)	r_{max}
10^{-3}	$3.9(\pm 0.32) \times 10^{-5}$ ($P < 0.001$)	0.463 ± 0.010 NS	$7.8(\pm 0.98) \times 10^{-7}$ ($P < 0.001$)	0.404 ± 0.010 ($P < 0.001$)
10^{-4}	$1.1(\pm 0.80) \times 10^{-4}$	0.481 ± 0.08	$3.3(\pm 0.83) \times 10^{-6}$	0.351 ± 0.022

Kinetic parameters for the stimulation of multiplication of N-HLF (WI-38, passage 20) and SV-HLF by Ca^{2+} and Mg^{2+} were determined for each ion as a function of the concentration of the other. The procedures were the same as described in Fig. 1. The concentrations of both ions were varied within a single series of assays using the same stock cultures, basal media and FBSP. Values $\pm 95\%$ confidence limits are indicated. The probability (P) that the difference between values is due to chance was determined by Student's t test and is indicated between rows. NS, not significant or $P > 0.10$.

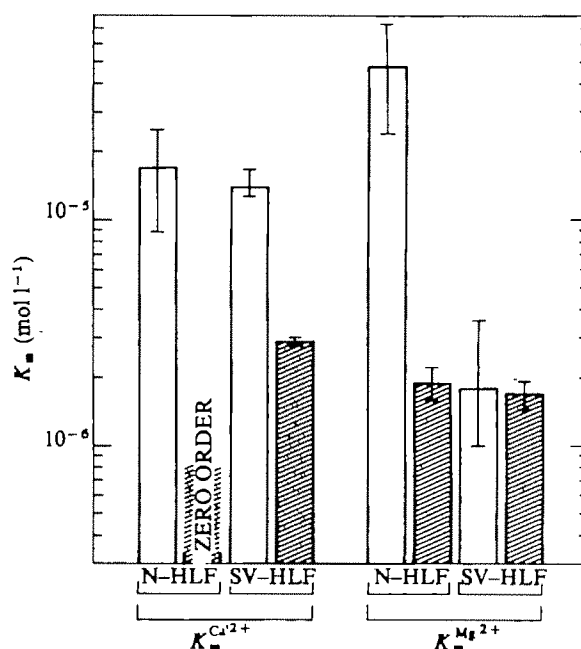
formation of CS can be treated as a first order function of S; (3) for each substrate or ligand (S), the rate of cellular multiplication (or survival) when all other substrates or ligands are constant is a first order function of the concentration of the CS complex; (4) the concentration of S is large relative to the concentration of sites in or on the cells, such that formation of CS does not significantly alter S; (5) consumption of S is sufficiently small that its concentration is not altered significantly and rates can be considered uniform throughout the assay period; (6) the rate of equilibration among cells, S, and CS complex is rapid relative to the rate of cellular multiplication or other processes that affect the concentration of CS; and (7) in the case of cellular multiplication, it is also necessary to assume that all daughter cells are identical to the parental cells, and cells remain in an exponential pattern of multiplication with no significant differentiation or other forms of withdrawal from the cell cycle.

The experimental system used in these studies is clonal growth of human diploid fibroblast-like cells derived from fetal lung (WI-38, MRC-5 and FLOW-2000 are interchangeable and are referred to as N-HLF) and a comparable transformed line (SV40 transformed WI-38, referred to as SV-HLF). An optimised medium (MCDB 105 (ref. 14)) was used which has each of its components adjusted to the centre of the concentration range yielding a zero order response¹²⁻¹⁴. The volume of medium per cell is large, and direct analysis of used media has verified that the concentrations of essential components do not change significantly during the assay period. Thus, the system seems to satisfy the assumptions made, as will be described elsewhere.

For N-HLF, both Ca^{2+} and Mg^{2+} are absolute requirements for multiplication, even when the other is present in saturating amounts. The requirements for multiplication are much greater than those for survival (Fig. 2). In these experimental conditions, the $K_m^{Mg^{2+}}$ for survival is only 5-6% of that for multiplication, and no effect of Ca^{2+} on survival rate can be demonstrated (zero order response). However, we cannot conclude that no Ca^{2+} is needed for survival, as atomic absorption spectrometry shows that the 'calcium-free' test medium contains $1.5 (\pm 0.12) \times 10^{-5}$ M calcium as a contaminant. The comparable 'magnesium-free' medium contains $8.6 (\pm 0.46) \times 10^{-6}$ M magnesium. These amounts are not due to contaminants in the high purity calcium and magnesium salts (each of which contains less than 2 p.p.m. of the other) or in the dialysed serum protein ($250\ \mu g\ ml^{-1}$) used in the assay.

Transformation of N-HLF with SV40 reduces the $K_m^{Mg^{2+}}$ for multiplication to that required for maintenance of either the normal or transformed cell (Fig. 2). In contrast, the amount of extracellular Ca^{2+} required for a half maximal rate of multiplication ($K_m^{Ca^{2+}}$) is changed little by transformation. However, some multiplication of SV-HLF occurs in the absence of added calcium, which could reflect either the background Ca^{2+} contamination or a 'basal' growth rate that does not require

Fig. 2 K_m values for multiplication and survival responses of N-HLF and SV-HLF to Ca^{2+} and Mg^{2+} . K_m values were determined as described for Fig. 1. Responses to Ca^{2+} and Mg^{2+} were measured with $250\ \mu g\ ml^{-1}$ FBSP and 1.0×10^{-3} M of the other ion. Survival rate was determined by incubating 100 cells per 60-mm Petri dish in 5.0 ml of a medium that is not permissive for multiplication. After 4 d, the medium was replaced with a complete growth medium and the dishes were incubated for a further 10 d after which the colonies were fixed, stained and counted. The survival media were similar to the growth media described for Fig. 1, except that in place of FBSP they contained $80\ \mu g\ ml^{-1}$ delipidated FBSP¹⁸. Delipidation of FBSP removes or inactivates one or more factors that are essential for multiplication of both types of cells¹⁹. N-HLF will survive in MCDB 105 without proliferation in the total absence of serum-derived factors^{13,14}. However, survival of SV-HLF in the same medium requires serum-derived factors that are not inactivated by delipidation. Delipidated serum was used with both types of cells for uniformity. Survival rate (number of survivors divided by days in survival medium) was measured as a function of concentration of Ca^{2+} or Mg^{2+} (with the other at 1.0×10^{-3} M) and K_m values for survival were determined from Lineweaver-Burke double reciprocal plots. White bars, multiplication; shaded bars, survival. Each value represents mean $\pm$ s.e.m. of three independent determinations using different stock cultures, media and FBSP preparations. Under the experimental conditions, Ca^{2+} has no effect on survival rate of N-HLF, possibly because of a low background level of calcium in the basal medium (see text).



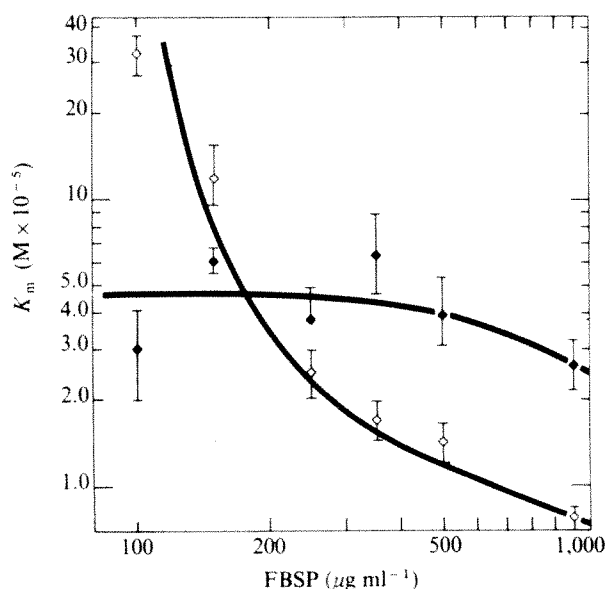
Ca^{2+} . Additional experiments in a cleaner background medium are needed to distinguish between these possibilities. In contrast to N-HLF, SV-HLF cells exhibit an easily measured survival response to Ca^{2+} which can be extrapolated to give a $K_m^{\text{Ca}^{2+}}$ of about 2.9×10^{-6} M. The $K_m^{\text{Mg}^{2+}}$ for survival is essentially unchanged by transformation.

In an attempt to determine whether Ca^{2+} or Mg^{2+} exerts the most proximal effect on cellular multiplication, we determined both K_m and $r_{\max}$ for each ion as a function of the other (Table 1). For N-HLF, the amount of Mg^{2+} in the medium has little effect on the amount of Ca^{2+} needed for a half maximal response ($K_m^{\text{Ca}^{2+}}$), but has a direct effect on the maximal growth response to calcium that can be obtained ($r_{\max}^{\text{Ca}^{2+}}$). Ca^{2+} , however, has no effect on the maximal growth that can be obtained ($r_{\max}^{\text{Mg}^{2+}}$), but low levels of calcium significantly increase the amount of magnesium needed for the response ($K_m^{\text{Mg}^{2+}}$). We interpret these results, in agreement with Rubin⁵, as indicating that for normal cells the role of Mg^{2+} is more proximal to the intracellular events that determine $r_{\max}$ for cellular multiplication than are those processes directly affected by Ca^{2+} .

In transformed cells, $r_{\max}$ values that can be achieved are affected less by Mg^{2+} , but seem to be dependent on Ca^{2+} . This observation is consistent with the interpretation that Mg^{2+} does not have a specific regulatory role in the multiplication of transformed cells and suggests that Ca^{2+} has the most proximal role in the multiplication of such cells. These striking and complex differences between normal and transformed HLF show the need for further investigation of the relationship.

In the presence of saturating amounts of Ca^{2+} and Mg^{2+} (10^{-3} M each), multiplication, but not survival, of N-HLF is dependent on serum macromolecules whose role is presumed to be regulatory¹⁴. To investigate the interactions among Ca^{2+} , Mg^{2+} , and serum-derived regulatory macromolecules, we determined K_m for both ions as a function of serum protein (FBSP) concentration in the medium (Fig. 3). K_m values for both ions tend to rise as FBSP concentration is reduced. However, at very low levels of FBSP, $K_m^{\text{Ca}^{2+}}$ rises very steeply, whereas $K_m^{\text{Mg}^{2+}}$ levels off, suggesting that Ca^{2+} may be the ion that interacts the more directly with serum macromolecules that exert multiplication-stimulating effects on normal cells.

Fig. 3 Effect of FBSP on apparent K_m for Ca^{2+} and Mg^{2+} for multiplication of N-HLF. Multiplication rate responses to Ca^{2+} and Mg^{2+} with the other at 1.0×10^{-3} M were measured at each of the indicated levels of FBSP and K_m values were determined in a single experiment as described in Fig. 1. The N-HLF stock culture used here was MRC-5 (passage 20). Vertical bars, s.e. due to the regression analysis. $\diamond$, $K_m^{\text{Ca}^{2+}}$; $\blacklozenge$, $K_m^{\text{Mg}^{2+}}$.



From these data we propose that (1) Ca^{2+} and Mg^{2+} have equally important roles in regulation of cellular multiplication that go beyond support of attachment and survival of non-proliferating cells; (2) transformation causes a selective loss of the regulatory role of Mg^{2+} , but not Ca^{2+} , in cellular multiplication; (3) in normal cells, Mg^{2+} has its regulatory effect on a process more proximal to the intracellular events of cellular replication than those processes affected directly by Ca^{2+} ; (4) in normal, but not transformed cells, the regulatory effect of Ca^{2+} is primarily mediated through Mg^{2+} -dependent processes; (5) the role of Ca^{2+} is more proximal than that of Mg^{2+} to the action of regulatory macromolecules from serum in the chain of events which ultimately determine multiplication rate of normal cells.

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Does Ca^{2+} cause fusion or lysis of unilamellar lipid vesicles?

MEMBRANE fusion is the central event in biological processes as diverse as secretion, fertilisation, endocytosis and pathological cell fusion, but as yet its mechanism is poorly understood¹. To elucidate this mechanism, bilayer phospholipid vesicles have been used extensively as models for interacting biomembranes^{2–5}. As a result of studies involving differential scanning calorimetry and freeze-fracture electron microscopy, it has been suggested that vesicles made from acidic phospholipids can fuse when exposed to divalent cations^{3,6}. For phosphatidylserine vesicles, the 'critical' Ca^{2+} concentration for membrane fusion seems to be ~ 1 mM, at which concentration a drastic increase in the permeability of the vesicle membranes has also been reported³. The techniques used to demonstrate 'fusion' in this system do not exclude molecular mixing of interacting membranes by mechanisms other than genuine fusion. In the latter case, the membrane-bounded internal compartments of two separate vesicles must become confluent in a process which transfers the contents of the initial vesicles to the resulting vesicle. It is possible, however, that Ca^{2+} may cause rupture of the lipid vesicles, with subsequent reassembly of the membrane fragments to form larger non-vesicular structures. If this is occurring, bilayer vesicles are poor paradigms for biological

Table 1 Leakage of ^3H -sucrose from liposomes

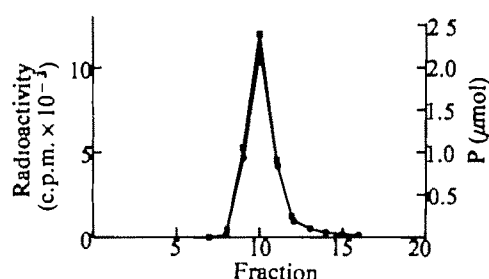
	Ca^{2+}	Mg^{2+}
Control	—	94.6
0.1	88.6	94.1
1	37.9	88.5
10	1.3	13.7

% of total radioactivity remaining associated with liposomes after incubation with divalent cations (mM) for 1 h. The higher divalent cation concentrations caused an increase in the size of lipid particles in the suspensions, preventing their passage through a Sephadex column. A Millipore filter (0.8 μm pore diameter) was mounted above the column and the sample to be eluted was pipetted on to it. The column eluant was treated similarly. Consequently, any lipid particles below the size limit of the filter appeared in the void volume and the eluant effectively washed any free sucrose from the larger particles trapped on the Millipore. Radioactivity remaining associated with the Millipore was counted after dissolving the filter in a Triton X-100-based scintillation mixture. A quenching correction was applied.

membrane fusion. One may predict the likelihood of vesicle fusion by calculating their energy of interaction due to electrostatic repulsion and electrodynamic attraction⁷. This approach shows that the addition of 1 mM Ca^{2+} to a suspension of phosphatidylserine vesicles is unlikely to reduce electrostatic repulsion between liposomes sufficiently for any but a fraction of the smallest ones to approach to 0.8 nm separation. This finding suggests that vesicles cannot approach closely enough to fuse and supports the notion that Ca^{2+} may initiate vesicle lysis and reassembly. We report here experimental support for vesicle lysis, based on the redistribution of a marker for the aqueous phase between vesicles and the external compartment on exposure to divalent cations.

Sonicated unilamellar vesicles were prepared from phosphatidylserine (Lipid Products, grade 1, >99% pure; stored before use in chloroform under nitrogen at -20°C) according to the methods of Papahadjopoulos *et al.*^{3,6,8-11}. The vesicles (10 μmol of lipid per experiment) were suspended in 2 ml of 100 mM NaCl containing 20 μCi of ^3H -sucrose (Amersham). One hour after sonication they were separated from untrapped sucrose by elution of the suspension through a Sephadex G-50 column. One 10-drop fraction (0.92 ml) representing the centre of the lipid peak was collected. This fraction was then exposed to a known final concentration of Ca^{2+} or Mg^{2+} for a further hour, after which the proportion of the total sucrose remaining associated with the lipid was estimated by eluting the sample from a second Sephadex G-50 column (the eluting medium contained divalent cations at the same concentration as in the sample) and determining the amount of radioactivity in each fraction using a liquid-scintillation counter. These amounts were then compared with the location of the lipid peak in the total elution profile, as determined by phosphate analysis (modification of the method described by Allen¹²).

Fig. 1 Elution of phosphatidylserine vesicles containing ^3H -sucrose from a Sephadex G-50 column (300 $\times$ 10 mm). The location of the lipid peak (■) was determined by phosphate analysis. Radioactivity (●) is expressed as c.p.m. $\times 10^{-3}$.



In the absence of divalent cations, most of the marker remained associated with the vesicles 1 h after the first gel filtration (Fig. 1). Exposure to increasing concentrations of Ca^{2+} or Mg^{2+} , however, brought about a progressive release of ^3H -sucrose from the liposomes (Table 1). Ca^{2+} was more effective than Mg^{2+} , a finding in agreement with the work of others¹³.

If this release was the result of a divalent cation-induced increase in vesicle membrane permeability, one would expect to be able to incorporate radioactive sucrose into previously unlabelled vesicles by exposing them to divalent cations. To test this, sonicated vesicles were prepared in the absence of ^3H -sucrose. 20 μCi of this radiochemical were then added to the samples immediately after sonication and the uptake of radioactivity into vesicles was assessed following exposure of the samples to Ca^{2+} or Mg^{2+} .

Without divalent cations, only 0.007% of the total marker became associated with the vesicles during the incubation (Fig. 2). Thus, very little sucrose had leaked into vesicles or been adsorbed on to their membranes. Furthermore, the presence of Ca^{2+} or Mg^{2+} hardly affected this uptake (Table 2). It might be argued that the results in Table 2 are artificially low due to the loss of vesicle-incorporated sucrose during the separation of the lipid from the external compartment by the filtration procedure (see legend to Table 1). To exclude this possibility, vesicles were prepared without ^3H -sucrose. This tracer was then added to the external compartment, followed by Ca^{2+} to a final concentration of 10 mM. After 1 h, non-incorporated sucrose was removed by gel filtration, eluting with divalent cation-free 25 mM EGTA.

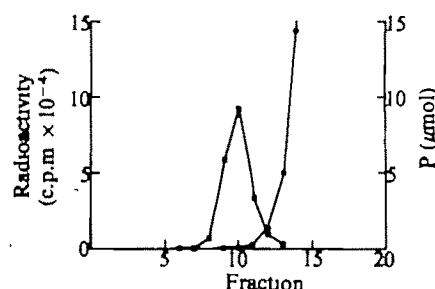


Fig. 2 Elution of a suspension of phosphatidylserine vesicles with ^3H -sucrose in the external aqueous compartment from a Sephadex G-50 column. Symbols as in Fig. 1.

Chelating agents can halt the Ca^{2+} -induced leakage of $^{22}\text{Na}^+$ and $^{42}\text{K}^+$ from phosphatidylserine liposomes¹³; thus, any ^3H -sucrose which had diffused into vesicles during incubation with 10 mM Ca^{2+} would not be washed out during elution. Only 0.002% of the total sucrose became associated with the lipid, implying that the values in Table 2 are not erroneously low.

Permeability coefficients for phosphatidylserine membranes may be calculated from Tables 1 and 2. In the 10 mM Ca^{2+} case, the ratio of these coefficients for release/uptake is 144:1 (1,170:1 in the EGTA version of the experiment). Divalent cations therefore seem to be able to bring about leakage of ^3H -sucrose from phosphatidylserine vesicles but not into them. It is unlikely that Ca^{2+} or Mg^{2+} is able to open a unidirectional pore in membranes as relatively simple as those under consideration. The most probable explanation for these findings is

Table 2 Leakage of ^3H -sucrose into liposomes

	Ca^{2+}	Mg^{2+}
Control	—	0.007
0.1	0.019	0.038
1	0.004	0.032
10	0.016	0.013

% of total radioactivity becoming associated with liposomes after incubation with divalent cations for 1 h. The % of total radioactivity that might be expected to be captured if the liposomal internal space equilibrated with the external space = 0.486. Divalent cation concentrations are in mM.

therefore that divalent cations bring about the complete rupture of phosphatidylserine vesicles, with production of structures which have lost the form of closed vesicles. This conclusion suggests the need for caution in interpreting vesicle 'permeability increases' under the influence of agents other than Ca^{2+} and Mg^{2+} . Regarding membrane fusion, it seems that the Ca^{2+} -phosphatidylserine system is an inappropriate model for biological membrane interaction, as the final product of the reaction between these components is not vesicular, although some initial fusion may occur on addition of Ca^{2+} to the suspension. Identification of the factors essential to fusion must await the development of a reliable assay for the occurrence of this process in artificial membrane systems.

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Uptake of charged lipid vesicles by isolated tomato protoplasts

LIPID vesicles act as carriers of biologically active materials into animal cells¹⁻³. A similar role might be envisaged for vesicles, for example, in the introduction of foreign DNA into plant protoplasts in genetic modification experiments⁴, or in the initiation of virus infection in plant protoplasts⁵. The amount of DNA which enters fed protoplasts is difficult to determine as is the multiplicity of virus which initiates infection in the poly-L-ornithine (PLO) (ref. 5) and polyethylene glycol (PEG) (ref. 6) procedures. To overcome these limitations of existing methods for the introduction of materials into protoplasts, I report here a vesicle system with the potential to fuse with the negatively charged plasmalemma⁷. Single membraned ('hollow') vesicles were considered more suitable to combine membrane instability with large 'load' volume, required to facilitate carriage of large molecules/virus particles into the protoplast and to release their content synchronously within.

Tomato protoplasts were isolated as previously described⁸. Positively charged vesicles were prepared as follows: equal volumes of 10 μM lecithin and 1 μM stearyl amine were dissolved in chloroform-methanol (4:1, v/v) and evaporated to dryness⁹. The residue was resuspended at 2.5 mg ml^{-1} in phosphate buffered mannitol (PBM, 12% w/v mannitol in 50 mM sodium phosphate buffer, pH 7.0) containing 0.01% w/v fluorescein diacetate and shaken vigorously by hand to give vesicles of about 0.002-0.006 μm diameter. These were loaded with fluorescein diacetate to facilitate study. The vesicles were collected by centrifugation at 50 g for 10 min. Vesicles were washed by resuspension, recentrifugation in PBM. Small vesicles remained in the supernatant and were discarded.

Protoplast and vesicle counts were made and the populations mixed in the ratio of 1:1, protoplasts:vesicles, in a volume of 1 ml (1×10^6 protoplasts) of PMB. Samples were examined

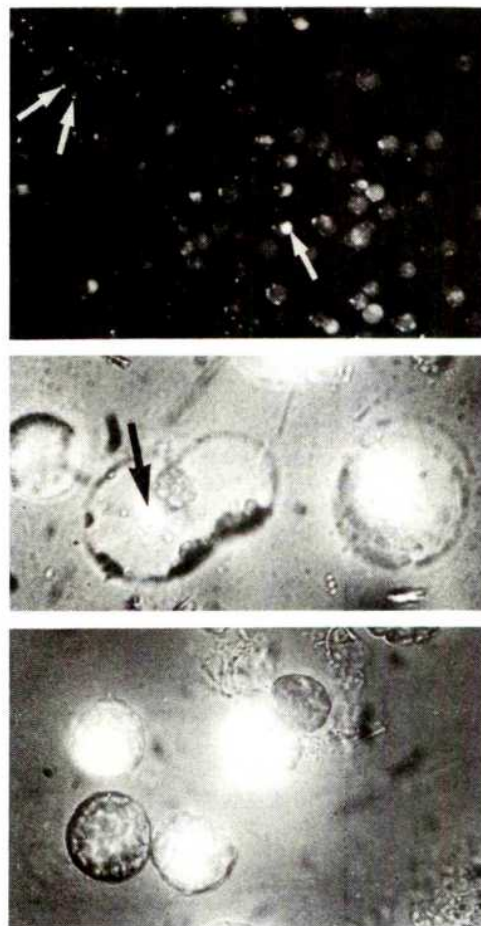


Fig. 1 Uptake of charged vesicles by isolated tomato protoplasts. Top, binding of vesicles to the plasmalemma, arrows indicate binding of one or more vesicles per protoplast. Middle, uptake of vesicles into the central vacuole, indicated by arrow. In the adjacent protoplast, the vesicle is releasing its contents. Bottom, some protoplasts showing intense fluorescence in the central vacuole following release of the vesicle contents.

immediately after mixing and at 15 and 25 h after mixing. Incubation was in the dark at 25 °C. A random sample of 181 protoplasts examined immediately after mixing showed a total of 140 vesicles bound (Fig. 1, top). After 15 h, vesicles were detected in the central vacuole (Fig. 1, middle), no vesicles were detected in the medium or still adhering to the outer surface of the plasmalemma, nor was fluorescence detected in the medium. After 24 h, vesicles were no longer detected, but the vacuoles of many protoplasts were fluorescing (Fig. 1, bottom).

Further work is in progress to prepare vesicles with less stable membranes than those described here, which on fusion with the plasmalemma may release their contents directly into the cytoplasm. The present study suggests that charged vesicles may provide a method of introducing quantifiable amounts of materials into plant protoplasts.

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Continuous production of monoclonal mouse IgE antibodies with known allergenic specificity by a hybrid cell line

SINCE the identification of IgE antibodies as mediators of allergic reactions by Ishizaka *et al.*¹ extensive work has been done to purify this new type of immunoglobulin from the serum of allergic patients and animals. However, all attempts so far have been limited by the very low serum concentration of these reaginic antibodies. This difficulty has been partly overcome with the detection of the extremely rarely occurring IgE-producing myelomas of the human² and the rat³, although IgE myelomas of other species are lacking. Moreover, none of the available IgE-myeloma proteins has a known allergenic specificity, which is indispensable for several important applications to future studies on the origin and mechanism of allergic processes. Here we describe a new murine cell line which is continuously producing high amounts of IgE antibodies with anti-ovalbumin (OA) specificity. This cell line was generated by cell fusion of murine myeloma cells with spleen cells from OA-hyperimmunised mice following the cell hybridisation technique developed by Köhler and Milstein⁴.

Table 1 PCA reaction of serum and ascites from IgE-14-205 tumour-bearing mice

Route of application	Time after inoculation (d)	Tumour rate	PCA titre Serum Ascites
Subcutaneous	8	5/5	8
	18	5/5	1,024
Intraperitoneal	8	4/5	32
	18	6/6	50,000 131,000
Intravenous	8	0/3	2
	22	2/3	3,000

Female BALB/c mice were inoculated with 5×10^5 hybrid cells from *in vitro* culture. log 2 dilutions of serum and ascites, 0.1 ml each, were injected intracutaneously at the back cover of male Wistar rats. After 48 h the animals were challenged with an intravenous injection of 0.5 ml 0.6% OA (5 times crystallised, Serva) in PBS containing 2% Evans blue. After 30 min the animals were killed. PCA titres are expressed as the reciprocal value of the highest dilution giving a PCA reaction.

The myeloma cell line P3-X63-Ag8, is an 8-azaguanine-resistant clone derived from the IgG1 (κ)-synthesising MOPC 21 myeloma of BALB/c origin. Spleen and mesenteric lymph-node cells for fusion were derived from female BALB/c mice immunised three to four times intraperitoneally at monthly intervals with 1–2 μ g of OA with Al(OH)₃ as described by Kulczycki *et al.*⁵. Three to seven days after the last immunisation spleen and mesenteric lymph-node cells were hybridised with P3-X63-Ag8 tumour cells at a ratio of 10:1 in the presence of 42% (w/v) polyethylene glycol (PEG), molecular weight 2,000 or 4,000, according to the method of Hämmerling⁶. The hybridised cells were resuspended in HAT medium (Dulbecco's modified medium supplemented with 10% heat-inactivated fetal calf serum, 5×10^{-3} M mercaptoethanol and hypoxanthine, aminopterin and thymidine⁷) and seeded at a density of 1×10^5 to 1×10^6 cells per well (serial dilutions) into multi-well tissue culture plates (Costar 3524). Because of the hypoxanthine guanine phosphoribosyl transferase (HGPRT)-deficiency of the P3-X63-Ag8 tumour cells, this HAT medium permits only growth of hybrid cells. Supernatants of wells containing hybrid clones were tested 3–5 weeks after cell fusion for IgE anti-OA antibodies, using the passive cutaneous anaphylaxis test of the rat (PCA) as described by Ovary⁸.

Table 2 Specificity for OA of antibody IgE-14-205

IgE supernatant	Allergen	PCA titre
+	OA	32
+	—	0
—	OA	0
+	BSA	0
Heat inactivated (2 h, 56 °C)	OA	0

For experimental detail see Fig. 1.

More than 2,000 individual hybrid cell lines were investigated. Only one of these was found to be positive in the PCA test. This hybrid cell line was found in a culture plate with vigorous cell growth in all wells, suggesting the presence of several hybrid clones in each.

The cells of this culture were therefore recloned several times by serial dilutions in Costar plates. A single PCA-positive clone from a culture plate with cell growth in less than 30% of all wells was chosen and recloned three more times by the same procedure, resulting in 100% PCA-positive subclones. A particular subclone (IgE-14-205) was selected for further propagation in spinner cultures.

The IgE anti-OA production was continuously controlled by the PCA reaction and it has now persisted for more than 8 months. The PCA titre of cell culture supernatants ranged between 1:64 and 1:256. The hybrid clone IgE-14-205 could also be propagated *in vivo* in adult BALB/c mice after subcutaneous or intraperitoneal inoculation of 5×10^5 hybrid cells (Table 1). Large quantities of IgE anti-OA antibodies were produced *in vivo*, especially by intraperitoneally growing tumours. The PCA titres ($>100,000$, see Table 1) by far exceeded those which can be reached by hyperimmunisation of mice.

The following findings demonstrate that antibody IgE-14-205 belongs to the IgE class and behaves like a typical murine reaginic antibody. (1) After heating to 56 °C for 2–4 h, IgE-14-205 cell culture supernatants had completely lost their skin-sensitising capacity (Table 2). This finding is consistent with the well known thermolability of IgE antibodies⁹, which is in contrast to all other immunoglobulin classes. (2) Sheep anti-rat IgE antiserum (Miles) in the Ouchterlony immunodiffusion test¹⁰ gives a single precipitation band against serum and ascites of IgE-14-205 tumour-bearing mice (data not shown). Moreover, the skin-sensitising capacity of IgE-14-205 cell culture supernatants could be removed by *in vitro* preincubation with this antiserum and subsequent centrifugation (Fig. 1). (3) Antibody IgE-14-205 shows no immunological crossreactivity

Fig. 1 Specificity of antibody IgE-14-205. PCA reaction of antibody IgE-14-205 was measured after preincubation of cell culture supernatants (24 h at 4 °C) with equal parts of log 2 dilutions of a, OA (1,000 to 32 μ g ml⁻¹ PBS); b, sheep anti-rat IgE (undiluted to 1:32 in PBS); c, BSA (1,000 to 32 μ g ml⁻¹ PBS). After preincubation the samples were centrifuged for 10 min at 10,000 r.p.m. and the supernatants were tested in the PCA. The PCA reaction was quantitated by extracting the blue skin plaques and measuring the extinction at 623 nm (= PCA units) as described by Spector¹¹. Each supernatant was tested in duplicate. The control supernatant was diluted 1:2 with PBS.

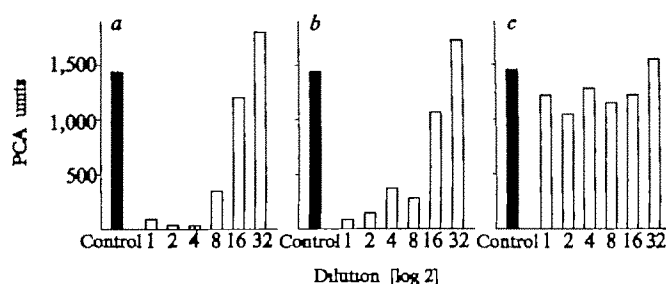


Table 3 Passive peritoneal anaphylaxis of the rat

Experimental group	No. of animals	Histamine release (IU)	(%)
Control with IgE	8	1.58 ± 0.31	100
Control without IgE	9	0.57 ± 0.05	0
5 mg per kg DSCG	5	0.74 ± 0.19	17

Male Wistar rats were injected intraperitoneally with 2 ml 1:5 diluted ascites from IgE-14-205 tumour-bearing mice (PCA titre 3,000). After 2 h 1 ml DSCG in PBS (5 mg per kg body weight) and 30 s later 5 ml OA in PBS (0, 4 mg ml⁻¹) containing 50 µg ml⁻¹ heparin were given intraperitoneally. After 5 min the animals were killed and the histamine content of the cell-free peritoneal exudate was estimated according to the method of Shore¹⁴. The animals of the negative control group received PBS instead of the IgE-ascites. IU, spectrofluorimetric intensity units.

with murine IgG2a, IgG2b, IgG3 and IgA (data not shown). It has a molecular weight of about 200,000, estimated by Sepharose-6B electrophoresis. (4) The allergenic specificity for OA of antibody IgE-14-205 was established by the demonstration that IgE antibodies from IgE-14-205 cell culture supernatants could be neutralised *in vitro* in a dose-dependent manner with OA but not with bovine serum albumin (BSA) (Fig. 1). In agreement with this finding is the observation that IgE-14-205 supernatants failed to produce a PCA reaction when either the related allergen (OA) was omitted or when an unrelated allergen such as BSA was used (Table 2). (5) When IgE-14-205 cell culture supernatants or serum and ascites from IgE-14-205 tumour-bearing mice were tested on rats, OA-specific PCA was observed. This result agrees with the well established cross reactivity of mouse and rat IgE antibodies with rat mast cells. Histologically, the PCA reaction in the rat was found to be a typical allergic reaction, with subcutaneous mast cell degranulation and dilatation of small blood vessels without any signs of a nonspecific inflammatory process. (6) Histamine release from peritoneal rat mast cells was induced in the passive peritoneal anaphylaxis test in rats¹² by serum and ascites from IgE-14-205 tumour bearing mice (Table 3). This allergic mediator release could be inhibited by anti-allergic drugs. Thus histamine release was almost completely blocked by 5 mg per kg body weight disodium cromoglycate (DSCG, Fisons) (Table 3).

In conclusion, the results described here confirm that the IgE-14-205 cell line represents a hybrid cell line which secretes OA-specific antibody of the IgE class. In addition to the reagenic antibody, the original hybrid cell line IgE-14-205 secretes the IgG1 (κ) immunoglobulin of the tumour P3-X63-Ag8, demonstrated in the Ouchterlony test with a rabbit anti-mouse IgG1 antiserum (Nordic). However, we have now selected two sublines which are highly active in secreting the reagenic antibody without any detectable IgG1 (κ) immunoglobulin in cell culture supernatants (data not shown).

Thus, the described hybrid cell line is the first tumour cell line which is continuously synthesising murine reagenic antibodies. It enables the production of murine IgE for various immunological, allergological and genetic studies, which have hitherto been extremely difficult¹³. Furthermore, antibody IgE-14-205 is the first available monoclonal IgE with known allergenic specificity. The murine hybrid cell line IgE-14-205 will be available on request exclusively for scientific purposes.

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Heavy HLA-DR (Ia) antigen chain is controlled by the MHC region

THE major histocompatibility complex (MHC), called the *HLA*-region in man and the *H-2* region in the mouse, contains many genes controlling an array of immunobiological phenomena. Thus certain parts of the immune response and various cell-cell interactions in normal immune processes are under genetic control from the MHC². Two structurally distinct groups of cell surface glycoproteins have so far been identified as products of genes localised to the MHC. The *HLA-A*, *B* and *C* loci control the expression of dimeric molecules composed of dissimilar subunits^{3,4}. The small subunit, β₂-microglobulin, is invariant and its structural gene resides on chromosome 15⁵. The large subunit carries the alloantigenic determinants which show that the *A*, *B* and *C* loci most probably harbour the structural genes for these polypeptide chains. The *HLA-D* locus also controls expression of cell surface antigens which are composed of two types of dissimilar subunits, and seem to be the human counterparts to murine Ia antigens⁶⁻⁹. Neither in man nor in the mouse have unambiguous data been provided demonstrating whether only one or both *HLA-D* (Ia) antigen subunits express the particular allotypic determinants. However, Barnstable *et al.*¹⁰ approached this problem by using the somatic cell hybridisation technique and provided evidence suggesting that at least the large, 35,000 molecular weight *HLA-D* antigen subunit is controlled by the MHC. In this note we demonstrate that by serological analyses and peptide mapping experiments the 35,000 MW *HLA-D* antigen chain is a product of the *HLA-D* locus.

HLA-D antigens were isolated from leukaemia cells of a patient suffering from chronic lymphatic leukaemia, as will be described elsewhere (L.K. *et al.* in preparation). The isolated *HLA-D* antigens were of high purity as revealed by SDS-polyacrylamide gel electrophoresis, gel chromatography, ultracentrifugation and immunological analyses. The *HLA-D* antigen phenotype of the patient, from whom cells were derived, was Dw3 and Dw4 as assessed by tissue typing techniques¹¹. The highly purified *HLA-D* antigens reacted with alloantisera directed against Dw3 and Dw4 but not with an alloantiserum directed against Dw2.

To establish which of the two *HLA-D* antigen subunits reacts with the alloantisera, ¹²⁵I-labelled¹² *HLA-D*-antigens were subjected to isoelectric focusing (Fig. 1). The material resolved into three main radioactive peaks, material from the peaks was extracted with a Tris-buffer containing Triton X-100 and aliquots were subjected to SDS-polyacrylamide gel electrophoresis¹⁴. The most acidic and basic fractions comprised the two *HLA-D* antigen subunits in highly purified form. The intermediate peak (c in Fig. 1) varied in size in different experiments. The observation that the amount of this material was inversely proportional to the amounts of the acidic and basic

components together with the SDS-polyacrylamide gel electrophoretic profile strongly suggest that the intermediate material (peak c) represents undissociated *HLA-D* antigen.

The separated subunits and the intact, radioactively labelled *HLA-D* antigens were treated with various alloantisera and heteroantisera in indirect immunoprecipitation assays¹⁵ to elucidate their antigenic properties. Table 1 summarises the results. The rabbit antiserum against *HLA-D* antigens¹⁵ reacted well with the intact *HLA-D* antigens as well as with the isolated subunits whereas the antiserum raised against *HLA-A, B* and *C* antigens³ did not react significantly better than normal rabbit serum. The alloantisera did not react as well with the intact *HLA-D* antigens as the rabbit antiserum, but it is obvious that each alloantiserum only recognised a subpopulation of the isolated *HLA-D* antigens as the combination of alloantisera precipitated about as much *HLA-D* antigens as the sum of the individual alloantisera. Alloantiserum directed against the specificity Dw2 did not react with the *HLA-D* antigens which

Fig. 1 Isoelectric focusing of highly purified ¹²⁵I-labelled *HLA-D* antigens. The labelled antigens were treated with 9 M urea in a buffer containing 2% Ampholines 3.5–10 (LKB) and 2% Triton X-100 for 2 h. The material was then subjected to isoelectric focusing¹³ in a 6% polyacrylamide gel containing 1%, pH 3.5–5, 1%, pH 5–8, and 0.5%, pH 3.5–10. Ampholines, 0.3 M sucrose, and 2% Triton X-100. After completed separation the gel was sectioned in an automatic gel slicer. Fractions containing radioactivity were pooled as indicated in the figure (b–d) mixed with 0.2 ml of 0.02 M Tris-HCl buffer, pH 8.0, containing 0.15 M NaCl and 0.5% Triton X-100, and gently agitated for 12 h to extract the radioactive material. Polyacrylamide was then removed by filtration over glass wool, and the radioactive materials were subjected to dialysis against the Triton X-100-containing Tris buffer. Aliquots from the three fractions were subsequently subjected to SDS-polyacrylamide gel electrophoresis¹⁴ (b–d). The arrows denote the migration positions of marker immunoglobulin G heavy and light chains and the tracking dye.

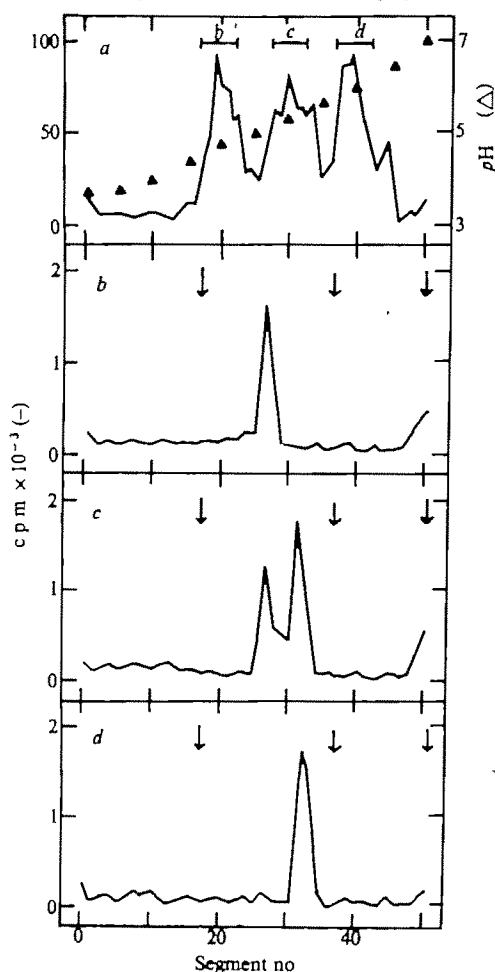


Table 1 Immunological reactivity of *HLA-D* antigens and their isolated subunits

Antiserum*	<i>HLA-D</i> antigens	28,000 MW	35,000 MW
		chain	chain
		% precipitated	
R α <i>HLA-D</i>	90	65	54
R α <i>HLA-A, B, C</i>	2	3	2
NRS	3	1	1
Dw3	34	4	16
Dw4	24	3	10
Dw3 + Dw4	52	3	21
Dw2	3	1	2
NHS	2	2	1

The ¹²⁵I-labelled *HLA-D* antigens and the separated subunits were incubated with 25- μ l portions of the alloantisera or 3- μ l portions of the rabbit sera as indicated above. After 5 h at room temperature immune complexes and free immunoglobulins were precipitated¹⁶. The precipitates were washed three times with ice-cold saline and subjected to SDS-polyacrylamide gel electrophoresis to establish subunit composition. The radioactivity in the precipitated proteins was measured. The data represent a typical experiment. Variations in the amount of radioactivity precipitated with the various antisera were noted in different experiments, although the data were qualitatively identical. Preliminary analyses suggest that the immune reactivity of the *HLA-D* antigens and the isolated subunits depends on the iodination conditions and the time spent in the urea-containing buffer.

The designations for the sera are: R α *HLA-D*, rabbit antiserum against *HLA-D* antigens; R α *HLA-A, B, C*, rabbit antiserum against *HLA-A, B, C* antigens; NRS, normal rabbit serum; Dw2, Dw3, Dw4, alloantisera against antigenic specificities Dw2, Dw3 and Dw4 respectively; NHS, normal human serum. The alloantisera were monospecific as determined by their reactivity in tissue typing experiments with use of the same target cells as used in the VII Histocompatibility Testing Workshop (Oxford). Their local designations are 3082B (Dw2), 4551 (Dw3) and 2503C (Dw4).

agrees with the results of the tissue typing (see above). Neither of the alloantisera recognised the 28,000 MW subunit whereas both antisera precipitated significant amounts of the 35,000 MW chain. The reactivity of the anti-Dw4 serum was considerably weaker than that of the anti-Dw3 serum. This observation is in part explained by the differences in titre between the two sera but the urea treatment used during the isolation procedure for the subunits may have deranged the Dw4-expressing *HLA-D* antigens more than the Dw3-expressing ones.

The lack of reactivity of the alloantisera against the 28,000 MW *HLA-D* antigen subunit may have depended on this chain being more sensitive towards denaturants than the 35,000 MW chain. This possibility had to be considered although the rabbit antiserum reacted well with the isolated 28,000 MW chain in as much as alloantigenic determinants may reside in limited parts of the chain. Further attempts to document an allotypic variation also for the 28,000 MW subunit were therefore made. ³H-Tyrosine-labelled *HLA-D* antigens were isolated by indirect immunoprecipitation¹⁶ using rabbit anti-*HLA-D* antigen serum. The sources of *HLA-D* antigens were the lymphoblastoid cell lines Daudi and Raji, which display the *HLA-D* phenotype Dw6/unidentified and Dw3/Dw6 respectively (C. Barnstable, personal communication). The *HLA-D* antigen subunits were separated by SDS-polyacrylamide gel electrophoresis. By running the electrophoresis in the absence of reducing agents a complete separation between the two subunits was obtained (compare ref. 7). The two types of subunits were separately extracted from the gel, digested with trypsin and subjected to peptide separation by high-pressure liquid chromatography. The sensitivity and reproducibility of this method makes it well suitable for the fractionation of radiochemical amounts of peptides and it has recently been used effectively for the isolation of peptides derived from trace amounts of *HLA-A* and *B* antigens (L.R., J.F. and P.A.P., in preparation). Figure 2 shows the results of the peptide separations. In analogy with the serological analyses described above

the 35,000 MW subunits derived from the Daudi and Raji cells displayed clearly discernible peptide differences. However, the peptide patterns recorded for the 28,000 MW chains were very similar. As can be seen in Fig. 2 the two types of *HLA-D* antigens subunits from a single cell line did not exhibit peptide patterns that argue for any obvious identity in primary structure. Since the procedure for the peptide mapping experiments was very reproducible it seems reasonable to conclude that the Daudi and Raji cell lines express very similar if not identical 28,000 MW *HLA-D* antigen subunits, whereas the 35,000 MW chains have different primary structures.

The data presented here, together with those of Barnstable *et al.*¹⁰, seem to establish that the large *HLA-D* antigen subunit by serological, structural and genetical evidence is derived from the MHC. By the same criteria there is no evidence to suggest that the small *HLA-D* antigen chain is coded for by the same genetic region. However, the I-region of the mouse controls the expression of *HLA-D* antigen analogues (the Ia antigens) from at least two loci, *A* and *E/C*¹⁷. Limited amino acid sequence analyses

demonstrated that both Ia-antigen subunits may be derived from the MHC^{18,19}. Further work is therefore needed to clearly demonstrate that the small *HLA-D* antigen subunit is not a product of the *HLA-D* locus.

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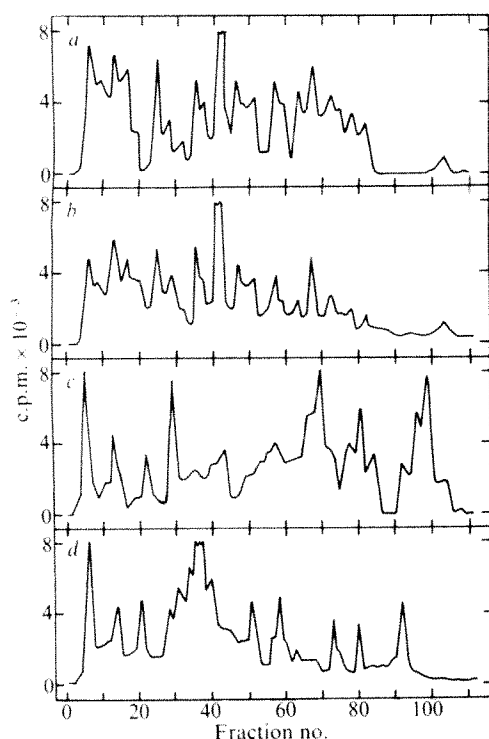


Fig. 2 Peptide patterns of ³H-tyrosine labelled *HLA-D* antigen subunits derived from Daudi and Raji cells. The labelled cells were washed, treated with 1% Triton X-100 in 0.02 M Tris-HCl buffer, pH 8.0, and the glycoproteins were isolated by affinity chromatography¹⁶. *HLA-D* antigens were isolated by indirect immunoprecipitation and immune complexes were separated by SDS-polyacrylamide gel electrophoresis¹⁴ in the absence of reducing agents. After segmentation of the gels the well-resolved *HLA-D* antigen subunits were extracted from the gel pieces essentially as described in Fig. 1. After the extraction remaining polyacrylamide gel was removed by filtration over glass wool. Human IgG (100 µg) was then added to each pool which was subjected to extensive reduction and alkylation. Excess reagents and SDS was removed by gel filtration on columns of Sephadex G-25 equilibrated with 0.02 M NH₄HCO₃. After lyophilisation the samples were taken up in 0.4 ml of 0.2 M NH₄HCO₃ containing 10 µg of trypsin. The digestions were terminated by lyophilisation after 3 h at 37 °C. The tryptic digests were finally dissolved in 50 µl of 0.05 M ammonium acetate, pH 4.5, containing 6% acetonitrile and subjected to high pressure liquid chromatography (Waters Associates). The C-18 column was operated at 50 °C. Elution was performed with a 60-ml linear gradient of acetonitrile, 6% to 40%, in the ammonium acetate buffer. Fractions of 0.3 ml were collected at 20 s intervals. a, Daudi 28,000 MW chain; b, Raji 28,000 MW chain; c, Daudi 35,000 MW chain; d, Raji 35,000 MW chain.

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9,11-Epoxyiminoprostano-5,13-dienoic acid is a thromboxane A₂ antagonist in human platelets

THROMBOXANE A₂ (TXA₂), an important arachidonic acid metabolite, aggregates platelets¹⁻⁶, constricts coronary smooth muscle^{1,7,8}, and is formed during mechanical or anaphylactic organ challenge⁹⁻¹¹. There are selective thromboxane A₂ synthetase inhibitors¹²⁻¹⁶ but there are no known, selective TXA₂-receptor antagonists. We report here that 9,11-epoxyimino-prostano-5,13-dienoic acid¹⁷ (9,11-EIP) antagonises TXA₂-mediated platelet aggregation without inhibiting thromboxane synthetase or fatty acid cyclo-oxygenase. This finding should allow further exploration of the nature of TXA₂ receptors in human platelets.

The fatty acid cyclo-oxygenase enzyme transforms arachidonic acid into prostaglandin (PG) endoperoxides (PGG₂, PGH₂)^{18,19}, which aggregate platelets either directly^{20,21} or after conversion to TXA₂^{1,4,22}. Platelet-rich plasma (PRP) and washed platelet suspensions containing 9,11-EIP were challenged with the following stimuli: arachidonic acid, PGH₂, TXA₂ generated *in vitro* by platelet microsomes⁴ and a sub-aggregatory dose of PGH₂, PG endoperoxide analogues^{23,24}, adrenaline or ADP. The aggregation profiles elicited by these agents were subsequently monitored. TXB₂ and PGE₂ concentrations during aggregation were quantitated by radioimmunoassay^{25,26}.

9,11-EIP suppressed TXA₂-induced aggregation of PRP in a dose-dependent manner (Fig. 1) with 50% inhibition occurring at <200 ng ml⁻¹. 9,11-EIP did not inhibit TXA₂ formation, because the TXA₂ was generated *in vitro* and added after its

formation. The results were identical in platelets treated with cyclo-oxygenase inhibitor (1×10^{-4} flurbiprofen), ruling out any contributions from the release of endogenous arachidonic acid metabolites. 9,11-EIP also antagonised aggregation induced by PG endoperoxide analogues^{23,24} (Fig. 2).

9,11-EIP suppressed PGH₂- and arachidonic acid-induced platelet aggregation in a dose-dependent manner at concentrations which did not affect TXB₂ levels (Fig. 3). PGE₂ and cyclic AMP levels were also unchanged (data not shown). Selective thromboxane synthetase inhibitors divert PGH₂ metabolism towards the formation of the bis-enoic prostaglandins, some of which, for example, PGD₂, can oppose aggregation by elevating cyclic AMP²⁵. Therefore, 9,11-EIP blocked aggregation independently of two common anti-aggregatory mechanisms, TXA₂ synthetase inhibition and cyclic AMP elevation²⁶. Results were similar in PRP. 9,11-EIP suppressed arachidonic acid- or PGH₂-induced aggregation in a dose-dependent manner at concentrations which did not affect TXB₂, PGE₂ or cyclic AMP levels. 9,11-EIP did not affect the first wave, but it suppressed the second wave of adrenaline- and ADP-induced aggregations, revealing its specific involvement with the arachidonic acid

Fig. 1 Effect of 9,11-EIP on TXA₂-induced aggregation of human PRP. PRP was prepared by centrifuging fresh, human whole blood (1 part 3.8% citrate/9 parts blood) at 200g for 10 min at 25 °C. TXA₂ was generated by incubating a sub-aggregatory concentration of PGH₂ (200 ng) with flurbiprofen-treated human platelet microsomes⁴ suspended in Tris buffer, pH 8.0 (20 µl containing 1 mg protein), for 20 s at 37 °C inside an aggregometer cuvette. PRP, preincubated at 37 °C for 2 min with 9,11-EIP was added to the cuvette containing TXA₂. The PRP was stirred at 1,100 r.p.m. at 37 °C and the aggregation profile was monitored with a Payton single-channel aggregometer. Increasing concentrations of 9,11-EIP suppressed TXA₂-induced aggregation in a concentration-dependent fashion. Effects from TXA₂ synthetase inhibition were precluded, as TXA₂ was generated *in vitro* before the addition of PRP and results were identical with PRP treated with 10^{-4} M cyclo-oxygenase inhibitors (aspirin, flurbiprofen). Results are consistent with TXA₂ antagonism. a, Control; b, 125 ng ml⁻¹; c, 250 ng ml⁻¹; d, 500 ng ml⁻¹; e, 2,500 ng ml⁻¹, 9,11-EIP.

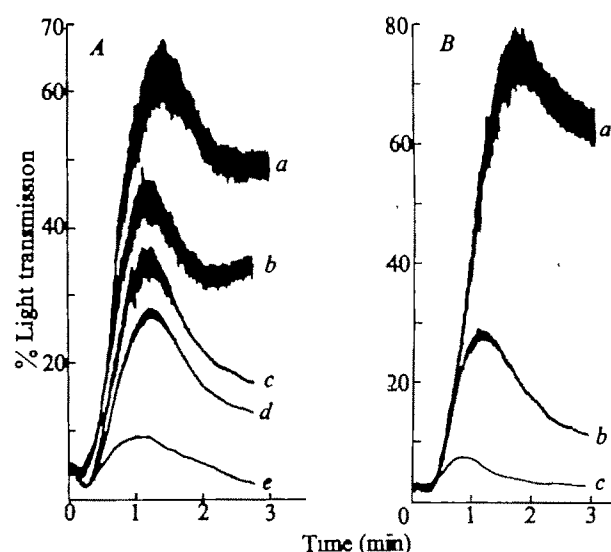
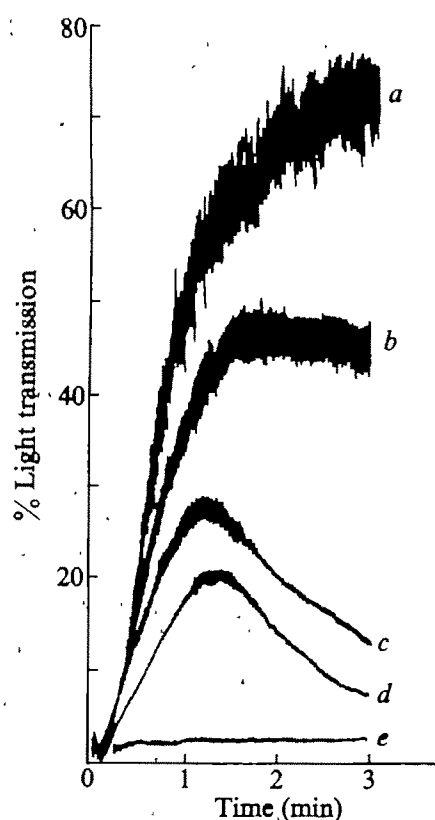


Fig. 2 Effect of 9,11-EIP on endoperoxide analogue-induced aggregation of human PRP. The conditions were similar to those of Fig. 1 except the endoperoxide analogues, 9,11-azido-15(S)-hydroxyprosta-5,13-dienoic acid (50 ng ml⁻¹) (A) or 9,11-methanoepoxy-15(S)-hydroxyprosta-5,13-dienoic acid (250 ng ml⁻¹) (B), were used so as to induce aggregation instead of TXA₂. The quantitatively different response of the antagonist against these analogues may reflect the fact that 9,11-azido-15(S)-hydroxyprosta-5,13-dienoic acid 'resembles' TXA₂ more than does 9,11-methanoepoxy-15(S)-hydroxyprosta-5,13-dienoic acid. A: a, control; b, 50 ng ml⁻¹; c, 100 ng ml⁻¹; d, 150 ng ml⁻¹; e, 300 ng ml⁻¹ 9,11-EIP. B: a, control; b, 250 ng ml⁻¹; c, 1.0 µg ml⁻¹ 9,11-EIP.

pathway. At concentrations exceeding 5×10^{-5} to 1×10^{-4} M, 9,11-EIP inhibited the platelet cyclo-oxygenase, reducing both TXB₂ and PGE₂ synthesis.

Needleman *et al.*²⁰ proposed that thromboxanes have at least two unique but dissociated properties, rabbit aorta constriction and platelet aggregation. The results with 9,11-EIP conditionally support this proposal. Although 9,11-EIP antagonises platelet aggregation, apparently by direct TXA₂-receptor blockade, it has intrinsic agonist properties for coronary smooth muscle, constricting the rat aorta and thereby preventing an assessment of any accompanying TXA₂ antagonism in this system. 9,11-EIP also inhibits PGI₂ formation in rabbit lung microsomes and BALB 3T3 cells at 10^{-5} M. Nevertheless, the thromboxane pathway dominates arachidonic acid metabolism in human platelets. TXA₂-receptor antagonists should further clarify the role of TXA₂ in platelet aggregation.

Conclusions on the role and mechanism of action of TXA₂ are frequently drawn by comparing the effects of selective inhibitors on the synthesis of arachidonic acid metabolites, modulation of cyclic AMP metabolism, and the attendant TXA₂-mediated physiology. As these inhibitors are only relatively selective³⁰, the conclusions are tentative and subject to re-evaluation. A TXA₂ antagonist is vital for further elucidation of the biochemical, pharmacological and physiological aspects of the arachidonic acid cascade in human platelets. 9,11-EIP is such an antagonist, with effects distinct from TXA₂ synthetase inhibition. Just as experiments with dichloroisoproterenol³¹ advanced the understanding of the β -adrenergic receptor, we believe that experiments with 9,11-EIP will advance the understanding of the TXA₂ receptor in platelets.

In summary, 9,11-EIP inhibited TXA₂-induced platelet aggregation in a dose-dependent manner. This inhibition was not due to an effect on TXA₂ synthetase because TXA₂ was generated *in vitro* before addition of PRP. 9,11-EIP also suppressed arachidonic acid- and PGH₂-induced aggregations of washed platelet suspensions and PRP without altering TXB₂,

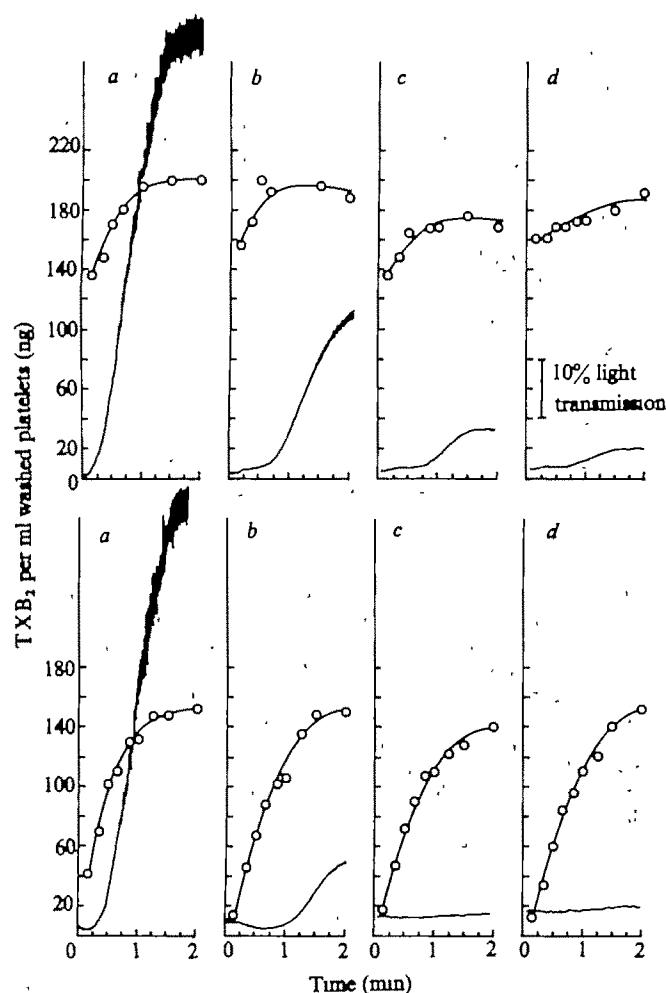


Fig. 3 Washed platelets were prepared according to Miller *et al.*²⁷. PGH₂ was prepared according to Gorman *et al.*²⁹. Platelet suspensions were incubated with 9,11-EIP for 2 min at 37 °C. The platelet suspension was then transferred to an aggregometer cuvette containing PGH₂ (0.20 µg) or arachidonic acid (1 µg) (upper graphs) or arachidonic acid (1 µg) (lower graphs). The suspension was stirred at 1,100 r.p.m. at 37 °C; aggregation profiles were monitored and samples (0.1 ml) were withdrawn for the radioimmunoassay of TXB₂. 9,11-EIP suppressed aggregation in a dose-dependent fashion without inhibiting TXA₂ or PGE₂ formation. The open circles represent TXB₂ levels. PGE₂ levels (data not shown) ranged over 0.10–0.50 ng ml⁻¹. Upper graphs, a, control; b, 0.40 µg ml⁻¹; c, 0.60 µg ml⁻¹; d, 1.0 µg ml⁻¹ 9,11-EIP. Lower graphs, a, control; b, 0.08 µg ml⁻¹; c, 0.80 µg ml⁻¹; d, 2.0 µg ml⁻¹ 9,11-EIP.

PGE₂ or cyclic AMP levels. These results are consistent with antagonism of TXA₂ in platelets at the receptor level. Note that others have discovered selective prostaglandin antagonists^{32–34}.

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Saccadic eye movements and visual stability

WE rarely confuse motions of our retinal images that result from movements of objects with those that result from eye movements. A predominant explanation of the perceptual stability that survives saccadic eye movements is that visual information about image movement is weighed against an internal ('extraretinal') signal about eye movement, and that a mismatch is generally perceived as movement of the world¹. Accurate information about eye position, even during a saccade, seems to be available to the oculomotor system^{2,3}, but it is not clear that this information finds its way to mechanisms underlying perceptual stability. The experiments we describe here show that errors in localisation following saccades are matched closely by errors in the size of saccades. We infer from this that the perceptual system does not monitor the extent of a saccade but merely assumes that an intended eye movement was made correctly.

The observer sits in a completely dark room with his head held steady by a dental impression. Ahead of him is an oscilloscope with an aluminised screen (P31 phosphor) on which appears a sharp, bright fixation spot (more than 2 log units above foveal threshold) and diameter 2–4 arc min, depending on viewing distance), with no surrounding glow. After 2.4 s this spot moves instantaneously a fixed distance to the left or right, where it remains for 100 ms before being extinguished. The direction of step is unpredictable to the observer, who tries to re-fixate the target, but this is extinguished before an eye movement can begin⁴ and so the saccade is made in the dark. 500 ms after extinction of the target, and after the saccade has been completed, a 'probe' spot appears for 500 ms at one of several fixed points about the target position, and the observer indicates with a keyswitch whether that spot lies to the left or to the right of the target.

This cycle is controlled by a computer and is repeated many times, with the direction of the target step and the position at which the probe spot appears, varying randomly from trial to trial. In a normal experimental session 350 trials are run in which the target steps to the right or left with approximately equal frequency, and for each direction of step the probe may appear in one of seven positions around the target position. From this we are able to plot two graphs, one for leftwards displacements, the other for rightwards, showing the relative frequency with which the probe in its various positions appeared to the left or right of the target. One such pair of graphs is shown in Fig. 1, for a target that stepped either 3.3° to the left (filled circles) or right (open circles). The smooth lines drawn through the points are the maximum likelihood estimates of the probit regression lines⁵

(the fits of which were rejected at the 10% level of significance in only three cases out of 21) from which one can obtain estimates of the mean and standard deviation of the distribution of judgements. The standard deviation of judgements is about 12 arc min, and the constant error, also about 12 arc min, is such that after a saccade the peripheral target seems to have moved further away from the fixation spot.

These errors, although small, are very reliable and indicate that in an impoverished environment the observer is not fully able to compensate for image displacements that result from saccades. The constant errors described here are a little smaller than those found by Monahan⁶ and Matin⁷ and much smaller than errors found by Bridgeman *et al.*⁸ in experiments to investigate perceptual mislocations related to saccadic eye movements; the task of subjects in the latter experiment was quite unlike that used here, which may explain the disagreement.

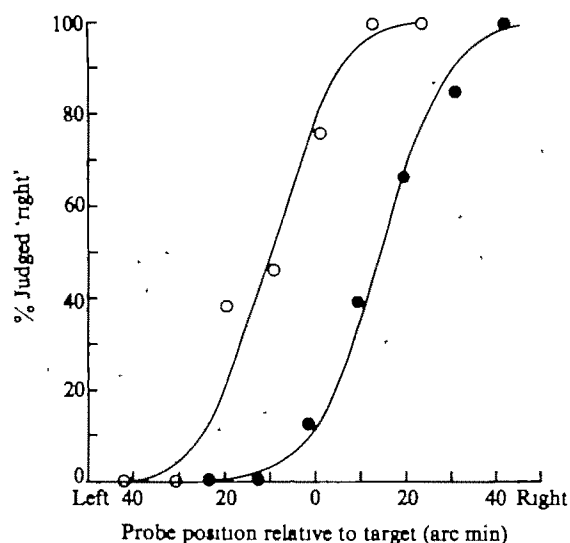


Fig. 1 Frequency with which the probe spot was judged to lie to the right of the target position (0 on the abscissa). The abscissa indicates probe position relative to the target. ● judgements made when a saccade followed a step of 3.3° to the left; O, corresponding judgements made following a step to the right. Smooth curves are maximum likelihood estimates of the probit regression lines. Each curve is based on an average of 175 trials in a single session, during which the probe appeared randomly at one of seven positions around the target point (observer P.L.).

Constant errors might have arisen because the initial target step was underestimated, although the saccades were made accurately to the (mislocated) target, or the saccade to the correctly located target may have been supposed accurate when in fact it fell short. Variability is probably due principally to two sources: first, the target is seen (initially) in peripheral vision, so its visual direction is to some extent uncertain. Moreover, the observer has to remember this direction during the 500 ms between target disappearance and the reappearance of a spot. Second, the extent of the eye movement to this uncertain, poorly remembered target may not be properly registered. The combined influence of these factors produces only small errors in localisation, a finding that might be interpreted as evidence for a reliable extraretinal signal that specifies the extent of the saccade, but an analysis of eye movements made during the experiment suggests otherwise. The horizontal position of the eyes was monitored (with a resolution of ± 4 arc min) by measuring the amount of infrared light reflected from slit images that lay horizontally over the corneo-scleral boundary, and a continuous record of eye position, together with a record of target position, was made on an oscillograph.

These measurements, a sample of which is shown in Fig. 2, indicate that at the time the probe appears the eye has stopped

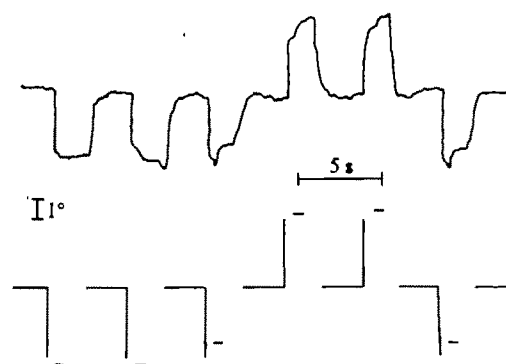


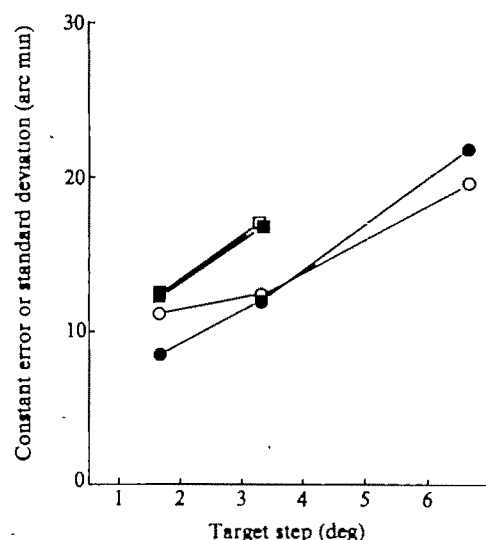
Fig. 2 Sample record of eye position during an experiment. The target stepped randomly 3.3° to the right or left and after 100 ms was extinguished. 500 ms later a probe appeared randomly at one of seven positions about the target point. The observer had to indicate whether the probe appeared to the right or left of the target. Corrective saccades occurred frequently after the probe appeared, usually so as to reduce an undershoot. Blank portions of the stimulus trace indicate that no spot was visible.

consistently short of the target by about 17 arc min, an amount close to that by which the target is mislocated. Moreover, the standard deviation of eye position (without allowance for error of measurement) is only a little greater (17 as opposed to 12.4 arc min) than that of judgements about target position.

Figure 3 shows that for steps in target position of 1.15° the mislocation is also close to the error in eye movement. Accurate measurement of eye movements to target steps of 6.6° was not possible, but errors in localisation follow the trend for smaller saccades.

Our results show that, following a saccade, the spot judged to lie in the target position is the one that falls on the fovea. Thus, to explain the small errors in locating targets after a saccade, we need only suppose that the observer relies on the eye movement being accurate and locates at the target position the spot that falls on the fovea. It is unnecessary to postulate an extraretinal signal that specifies accurately the size of the saccade; clearly,

Fig. 3 Accuracy and reliability of judgements about target location following saccades to target steps of different sizes (circles). Both parameters were estimated from probit regression lines. Points are averages for target steps to left and right, and a positive constant error indicates that the probe judged to lie on target was too close to the original fixation point. Accuracy and reliability of saccades to target steps of different sizes (squares). Points are averages for saccades to left and right. Open symbols, standard deviation; filled symbols, constant error.



some signal is needed to indicate that the eye has moved, but more precise information is not required.

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Vanadate-stimulated natriuresis

THE observation^{1–3} that V(v) is a potent inhibitor of (Na⁺, K⁺)-activated renal ATPase raised the possibility that it has natriuretic and thus diuretic properties in the living animal. We report here experiments showing that it has.

Plastic catheters were placed in the urinary bladder and femoral vein of rats (250–350 g) under ether anaesthesia. While recovering from the anaesthetic, the animal was confined in a restraining box and placed on a laboratory balance. The opposite arm of the balance actuated the delivery of isotonic saline (145 mM NaCl, 5 mM KCl) from a peristaltic pump to the femoral vein, in such a way that, during the experiment, the urinary and evaporative losses were made good and the weight of the animal was maintained.

During the succeeding 2 h the rate of flow of urine became roughly constant. Sodium orthovanadate (1.5 µmol) was then injected intravenously in 0.15–0.3 ml of the same isotonic saline; these volumes are too small to affect urine flow by augmenting the plasma volume. Table 1, which summarises the results of four consecutive experiments, shows the total excretion of water, sodium and potassium during the hours immediately preceding and immediately following the administration of vanadate. The flow of urine increased dramatically within a few minutes of the vanadate injection, reaching peak rates of about 1 ml min⁻¹. As this represents approximately 50% of the glomerular filtration rate⁴, it is likely that vanadate acts, at least in part, on the proximal tubule. The sodium

Table 2 Distribution of ⁴⁸V 75 min after the intravenous injection of 1.5 µmol ⁴⁸V-vanadate

Tissue	Blood plasma	Renal cortex	Renal outer medulla	Renal papilla	Urine
⁴⁸ V content (µmol per kg wet weight)	14.9	62.0	10.2	7.8	2.6

concentration in the urine produced in response to vanadate approximated to that in plasma. The integrity of the nephrons was not lost, for neither glucose nor protein was detectable in any of the samples of urine. Also, the effect of vanadate seems to be reversible, as, in longer-term experiments, urine flow following a vanadate injection diminished after a few hours, but the kidney was still capable of responding to a further dose as it had before.

In the first of the experiments included in Table 1, we used ⁴⁸V-labelled vanadate and determined the distribution of tracer in the kidney and elsewhere 75 min after its administration, when the diuresis was still nearly maximal. Table 2 shows that ⁴⁸V was selectively concentrated in the renal cortex. Otherwise, the distribution of radioactivity in the various organs was similar to that described by Hathcock *et al.*⁵. Within 75 min of the ⁴⁸V injection, 43% of the radioactivity had been excreted.

We conclude that vanadate is a potent natriuretic and diuretic substance, perhaps the most potent now known. It remains to be determined (1) whether vanadate, at the naturally occurring blood or tissue levels, has a physiological role in controlling natriuresis, and (2) whether the natriuretic and diuretic effects of vanadate can be therapeutically useful.

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Table 1 Excretion of water, sodium and potassium before and after the intravenous injection of sodium orthovanadate

Experiment	Excretion before injection			Excretion after injection		
	Water (ml h ⁻¹)	Na (mEq h ⁻¹)	K (mEq h ⁻¹)	Water* (ml h ⁻¹)	Na (mEq h ⁻¹)	K (mEq h ⁻¹)
1	5.22	0.598	0.325	49.6	7.05	0.442
2	1.53	0.212	0.267	38.7	6.10	0.333
3	1.43	0.175	0.127	27.9	2.53	0.313
4	2.10	0.220	0.197	28.5	3.91	0.301
Mean ± s.e.m.	2.57 ± 0.90	0.01 ± 0.099	0.299 ± 0.043	36.2 ± 5.1	4.84 ± 1.07	0.347 ± 0.032

The dose, 1.5 µmol, was chosen arbitrarily as that which, if it were distributed uniformly in the body water, would give a concentration sufficient *in vitro* to cause 75% inhibition of (Na⁺, K⁺)ATPase prepared from pig kidney. 1.5 µmol vanadate in a 300 g animal represents about 2.5% of the LD₅₀, determined in mice⁶; estimates of LD₁₀₀ suggest that vanadate is about equally toxic to rats and mice⁶.

* For comparison, it is worth noting that an equimolar dose of the therapeutic diuretic furosemide injected intravenously into a 300 g rat caused a diuresis averaging about 10 ml h⁻¹ during the first hour, with peak flow rates up to about 0.3 ml min⁻¹.

Peptidyl-transfer RNA hydrolase prevents inhibition of protein synthesis initiation

PEPTIDYL-tRNA HYDROLASE (called hydrolase below) catalyses the hydrolysis of peptides from peptidyl-tRNAs that are not bound to ribosomes^{1,2} but will not hydrolyse amino acyl-tRNA or fMet-tRNA_{fMet}. It is interesting that hydrolase seems to be ubiquitous in nature; it has been found in all cells where it has been sought, including yeast³, bacteria, rat liver and jackbeans⁴. However, the metabolic role of this enzyme has not been established. It is required for normal function, as a mutant strain of *Escherichia coli* with a temperature-sensitive hydrolase cannot continue protein synthesis at the nonpermissive temperature⁵. Hydrolase itself is not required for initiation, elongation or termination phases of protein synthesis⁶. Menninger⁷ has shown that peptidyl-tRNA, formed on ribosomes, accumulates at nonpermissive temperatures in cells with the temperature-sensitive hydrolase. Hydrolase thus seems to be a scavenger enzyme of peptidyl-tRNAs normally aborted from ribosomes during protein synthesis. Of interest now is why peptidyl-tRNA is frequently prematurely released from ribosomes and why protein synthesis is inhibited in the mutant strain with a defective hydrolase⁵. I report here work on the latter problem and present evidence which indicates that peptidyl-tRNAs accumulate and compete with *N*-formyl-Met tRNA_{fMet} during initiation of protein synthesis.

Two possible mechanisms of protein synthesis inhibition at 42°C in the temperature-sensitive hydrolase strain are that one or more infrequently occurring tRNAs may become sequestered as peptidyl-tRNA, resulting in inhibition of polypeptide elongation or, on the other hand, that the only known product of the reaction (peptidyl-tRNA) may interfere with initiation of polypeptides. Peptidyl-tRNA is not unlike *N*-formyl-Met-tRNA_{fMet}. Thus, these two similar compounds may compete at initiation for the P-site on the ribosome.

When the hydrolase mutant strain is incubated at temperatures intermediate between permissive growth and nonper-

missive growth (40, 40.5, 41 °C), partial inhibition of growth is observed. This is interpreted as limited inactivation of hydrolase, resulting in a reduced rate of peptidyl-tRNA hydrolysis. If elongation rates are affected at these intermediate temperatures, protein polymerisation time should be increased. If initiation of protein synthesis is inhibited, however, the polymerisation rate will remain unchanged for a specific protein.

The time lag between the induction of the lactose operon and the first appearance of β -galactosidase activity above basal enzyme level was used to estimate the peptide chain elongation rate^{8,9}. It has been shown that this approach is not a reflection of changed mRNA synthesis times⁸. Hence a measure of polymerisation time of β -galactosidase can differentiate between the two possibilities outlined above.

The data presented in Table 1 indicate that the rate of peptide-chain elongation does not change significantly with increase in growth temperature in the hydrolase temperature-sensitive strain. The chain elongation rate is slightly less in the mutant strain at 40 °C than in the parent strain (20%), but nearly identical at the permissive temperature (30 °C). However, the growth rate of the mutant strain decreases considerably as the growth temperature is increased from 37 to 41.5 °C, reflecting inhibition of protein synthesis by steadily increasing concentrations of peptidyl-tRNA in the cell. Furthermore, the relative rate of polypeptide chain initiation is proportionally decreased (Table 1, column 5). These data are derived from the initial slope of the induction curve; they reflect the relative number of molecules synthesised per unit time. The relative number of chain initiations in strain AA-7852 (*ptf*⁻) decreases by slightly more than half from 40 to 41.5 °C, and the doubling time decreases proportionally. No significant growth or β -galactosidase induction is observed at 42°C, reflecting complete inactivation of the hydrolase.

Other data suggesting that initiation of protein synthesis is inhibited in the hydrolase mutant strain at the nonpermissive temperature come from studies of the polysome distribution after shift to 43 °C. Menninger *et al.*⁶ found that polysomes slowly dissociated at 43 °C, forming 70S subunits. Elongation continues temporarily, resulting in dissociation of polysome structures. However, initiation only forms 70S units, possibly because of a peptidyl-tRNA in the P-site. This is consistent with the idea that some poison accumulates, very probably peptidyl-tRNA, but must reach a critical level before inhibition is seen. Protein synthesis is not inhibited in the hydrolase mutant until about 10 min after transfer to the nonpermissive temperature.

In conclusion these data show that the number of polypeptide chain initiations decreases as hydrolase is partly inactivated. On the other hand, the chain elongation rate is not affected. These findings imply that hydrolase plays an important part in cell metabolism by destroying spontaneously formed peptidyl-tRNAs which, in turn, inhibit protein synthesis initiation, probably by competing with *N*-formyl-Met-tRNA_{fMet} in the P-site of the ribosome.

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Table 1 Amino acid polymerisation and growth rates at various temperatures

Strain	Growth temperature	Doubling time (min)	Polymerisation rate (amino acids s ⁻¹)	Initial slope of induction curve (nmol ONPG hydrolysed per min per 3 × 10 ⁸ cells)
AA-7852	30.0 ± 0.05	190 ± 5	5.6 ± 0.3	55
	37.0 ± 0.05	170 ± 5	6.8 ± 0.2	57
	40.0 ± 0.05	210 ± 8	7.5 ± 0.2	42
	41.0 ± 0.05	270 ± 10	7.3 ± 0.2	29
	41.5 ± 0.05	300 ± 10	7.3 ± 10	21
CP-78	30.0 ± 0.05	150 ± 5	6.1 ± 0.3	72
	40.0 ± 0.05	110 ± 5	9.1 ± 0.2	75

Cells were grown in M-9 medium with 0.4% glycerol and required amino acids in a gyratory shaking water bath at the specified temperature⁶. Strains CP-78 (a K12 strain of *E. coli*, *arg*, *his*, *leu*, *thr*, *thi*) and AA-7852 (a single-step mutant of strain CP-78 that is now temperature-sensitive for peptidyl-tRNA hydrolase (ref. 5) were induced for β -galactosidase synthesis by addition of 5 mM 3', 5'-cyclic AMP and 1 mM isopropylthiogalactoside after growth at the specified temperature for 2 hours. The cultures were sampled every 5 s by taking 0.5 ml into test tubes containing 0.50 ml of a solution (1 mg ml⁻¹) of chloramphenicol in distilled water⁸. These samples were assayed for β -galactosidase activity by cell lysis with toluene and hydrolysis of o-nitrophenol- β -D-galactopyranoside (ONPG) at 25 °C. Polymerisation rate was established by the time required for the first newly synthesised β -galactosidase molecules to appear^{8,9}. There are 1,021 amino acids in β -galactosidase¹⁰. A change of 0.004 A₄₂₀ units equals 1 nmol of ONPG¹¹.

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Inhibition of Q β RNA 70S ribosome initiation complex formation by an oligonucleotide complementary to the 3' terminal region of *E. coli* 16S ribosomal RNA

It has been proposed that a key event in the recognition of a prokaryotic protein initiation site by the ribosome is the interaction between the 3'-terminal sequence of the 16S rRNA and a purine-rich sequence preceding the initiator triplet by three to nine nucleotides^{1,2}. This hypothesis predicts that if the 3'-terminal region of the 16S rRNA were blocked, binding of the ribosome to the initiator region of an mRNA should be diminished or prevented, while binding to the trinucleotide A-U-G should not be affected. We have shown that the oligonucleotide A-G-A-G-G-A-G-G-U_{OH} (P-2a), eight bases of which are complementary to the sequence A-C-C-U-C-C-U-U-A_{OH} at the 3'-terminal region of 16S rRNA ($\Delta G = -16.9$ kcal mol⁻¹ calculated as described in Table 1), binds tightly to the 30S ribosome and is released as a complex with the 3'-terminal 49-mer of 16S rRNA after digestion with cloacin DF13 (ref. 3). We report here that this oligonucleotide either strongly inhibits Q β RNA binding to 70S ribosomes or

decreases the stability of the initiation complex formed in its presence. As we found that the formation of complexes of pA-U-G and 70S ribosomes was not inhibited by the oligonucleotide, we conclude that inhibition (or destabilisation) of Q β RNA binding is not due to an effect on the interaction between fMet-tRNA and the initiation triplet or on the association of the 30S and 50S ribosome subunits.

Incubation of the low-salt-washed ribosomes (which retain initiation factors) and intact Q β RNA in the presence of fMet-tRNA leads to the formation of a complex sedimenting at 70S (ref. 4). As in the case of all phage RNAs, ribosomes attach predominantly to the initiation site of the coat cistron, and to a lesser extent to that of the replicase cistron⁴⁻⁷.

Table 1 and Fig. 1 show the effect of preincubating ribosomes (5 pmol) with different amounts of purified P-2a before the addition of ³H-labelled Q β RNA (2 pmol). It is seen that the amount of Q β [³H]RNA in the 70S peak is about 0.4 pmol (20% of the input) in the absence of P-2a (Fig. 1, left figure) and decreases with increasing amounts of P-2a. At a molar ratio of P-2a to Q β RNA of 2.5, 50% inhibition of 70S complex formation was observed, and at a ratio of 10, inhibition was 87% (Fig. 1, right figure, Table 1). The effect of several other oligonucleotides on 70S complex formation was compared to that of P-2a in the experiment in Table 1. An equimolar mixture of P-2a and P-2b (which are difficult to separate) had the same inhibitory effect as pure P-2a.

To show that inhibition of complex formation was not due to an inhibitor other than an oligonucleotide, an aliquot of the mixture of (weakly ³²P-labelled) P-2a and P-2b was treated with Sepharose-linked RNase T₁; the equivalent of 20 pmol of the ³²P-labelled oligonucleotides, which after this treatment were completely digested (as shown by electrophoretic analysis) gave virtually no inhibition (Table 1). The RNase A-resistant oligonucleotides G-G-A-A-G-A-G-A-G-C_{OH} (P-4) and G-G-A-G-A-A-G-C_{OH} (P-6), which showed less complementarity with the 3'-terminal region of 16S rRNA (Table 1) than P-2a, were far weaker inhibitors; the concentrations required to give 50% inhibition were about three and five times greater, respectively,

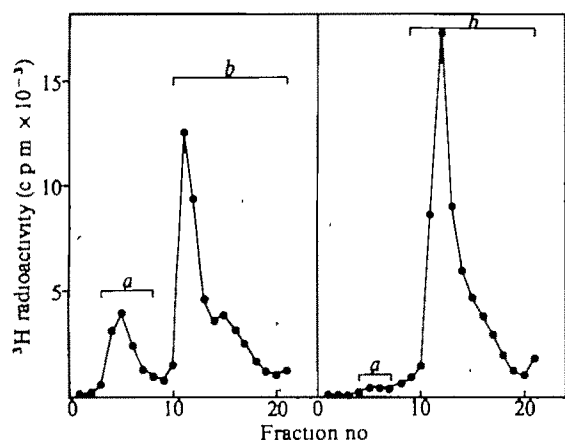


Fig. 1 Effect of oligonucleotide P-2a on binding of Q β [³H]RNA to 70S ribosomes. The preparation of the pancreatic oligonucleotide P-2a will be described elsewhere³. Q β [³H]RNA, fMet-tRNA and ribosomes were prepared as described previously¹⁴. Low-salt-washed ribosomes (5 pmol) were incubated with or without P-2a (20 pmol) in 50 mM Tris-HCl (pH 7.5), 6 mM MgCl₂, 120 mM NH₄Cl, 0.5 mM GTP, 0.5 mM dithiothreitol and about 440 pmol of unfractionated, stripped tRNA charged with fMet (ref. 14) (estimated to contain 10 pmol of fMet-tRNA) in a volume of 10 μ l for 15 min at 37°C. Then, 2 pmol of Q β [³H]RNA (specific activity, 3×10^4 c.p.m. pmol⁻¹; in 2 μ l) were added to the reaction mixture and incubation was continued for 10 min at 37°C. 100 μ l of buffer B (10 mM Tris-HCl (pH 7.5), 10 mM MgCl₂, 50 mM NH₄Cl and 10 mM β -mercaptoethanol) were added and the sample was loaded on a 4 ml 10–30% sucrose gradient in buffer B. Centrifugation was for 90 min at 58,000 r.p.m. and 4°C in an SW60 Spinco rotor. 21 Fractions were collected on paper disks (Schleicher and Schuell, LS78 Ruffi, diameter 22 mm) and ³H radioactivity was determined in POPPOP-scintillator. The A₂₆₀ profile of a mixture of 30S, 50S and 70S ribosomes, run in the same conditions, was used to locate the ³H radioactivity sedimenting at 70S (indicated by a). The amount of 70S ³H radioactivity was calculated relative to the total ³H radioactivity recovered from the gradient (a + b). Left hand figure, without P-2a, right, with P-2a (20 pmol).

Fig. 2 Sedimentation analysis of complex formation between ribosomes and Q β [³H]RNA and [³²P]pA-U-G: effect of fMet-tRNA and oligonucleotide P-2(a+b). The reaction mixture contained 92 mM Tris-HCl (pH 7.5), 0.18 M NH₄Cl, 9.2 mM MgCl₂, 0.92 mM dithiothreitol, 0.92 mM GTP, 10 pmol of low-salt-washed ribosomes, oligonucleotide as indicated below and about 1.3 nmol of stripped, unfractionated tRNA loaded with fMet (estimated to contain about 30 pmol fMet-tRNA) in a volume of 13 μ l. After 15 min at 37°C, 2 pmol of Q β [³H]RNA (○) (specific activity, 1.3×10^5 c.p.m. pmol⁻¹) and 100 pmol of [³²P]pA-U-G (●) (specific activity, 1.9×10^4 c.p.m. pmol⁻¹) were added in 7 μ l and incubation was continued at 37°C for 10 min. The sample was then analysed essentially as described in Fig. 1. a, No oligonucleotide, plus fMet-tRNA; b, no oligonucleotide, no fMet-tRNA; c, 40 pmol of P-2(a+b), plus fMet-tRNA. Brackets indicate the fractions corresponding to the 70S complex.

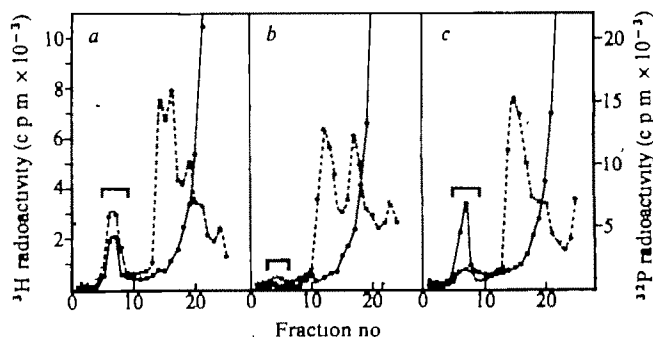


Table 1 Inhibition of binding of 70S ribosomes to Q β RNA by oligonucleotides

Oligonucleotide	Nucleotide sequence (ΔG (kcal mol ⁻¹))*	Amount added (pmol oligonucleotide)	% of Q β [³ H]RNA bound to 70S ribosomes (relative to control†)	% Inhibition
None		none	100	0
P-2a (purified by ribosome binding) [‡]	A-G-A-G-G-A-G-G-U _{OH} (-16.9)	5	42.6	57.4
P-2a (purified by ribosome binding) [‡]		10	32.6	67.4
P-2a (purified by ribosome binding) [‡]		20	12.8	87.2
P-2(a+b) (1:1 mixture)	A-G-A-G-G-A-G-G-U _{OH} (a) (-16.9) G-G-G-A-G-A-A-G-U _{OH} (b) (-10.0)	20	8.8	91.2
P-2(a+b), treated with RNase T ₁ †		20	115.0	0
P-4	G-G-A-A-G-G-A-G-C _{OH} (-11.1)	20	43.4	56.6
P-6	G-G-A-G-A-A-G-C _{OH} (-7.9)	20	57.5	42.6
T-14	A-U-U-U-C-U-U-C-U-A-U-G _{OH} (-1.1)	20	105.0	0
T-2	A-U-A-U-U-A-U-U-U-A-C-C-G _{OH} (-1.1)	20	90.0	10.0

Low-salt-washed ribosomes (5 pmol) were incubated with oligonucleotides in the conditions described in the legend to Fig. 1 for 15 min at 37 °C, then Q β [³H]RNA (2 pmol, specific activity 6.9 $\times 10^4$ c.p.m. pmol⁻¹ or 3 $\times 10^4$ c.p.m. pmol⁻¹) was added to the mixture and incubation continued for 10 min at 37 °C. The mixture was diluted with 100 μ l of buffer B and analysed by 10–30% sucrose gradient centrifugation as described elsewhere⁵.

* Free energy of optimal interaction with the sequence HOA-U-U-C-C-U-C-C-A-C-U-A-G (5') of the 3' end of 16S rRNA was calculated according to Gralla and Crothers¹⁷, without considering the free energy contribution resulting from the bimolecular nature of the reaction.

† In the control experiment where oligonucleotide was omitted, about 20% of the input Q β [³H]RNA was bound to 70S ribosomes (see Fig. 1, a).

‡ BrCN-activated Sepharose 4B was prepared and RNase T₁ was immobilised essentially as described¹⁵. ³²P-labelled oligonucleotide P-2(a+b) (50 pmol in 5 μ l H₂O; specific activity, 200 c.p.m. pmol⁻¹) was allowed to run into a small column (0.1 ml) of immobilised RNase T₁ and to stand for 15 min at room temperature. The RNA was rinsed out of the column and an aliquot was taken for electrophoretic analysis¹⁶; only A-A-Gp, A-Gp, Gp and Up were detected. Digested RNA equivalent to 20 pmol of P-2(a+b) (as determined by radioactivity) was tested for inhibitory activity as described for the untreated fragments.

than those required with P-2a. Two pyrimidine-rich RNase T₁-resistant oligonucleotides, T-14 and T-22, gave virtually no inhibition at a molar ratio of 10 relative to Q β RNA (Table 1). It is interesting in this connection that AG-rich random copolymers were found to be potent inhibitors of ribosome binding to natural mRNA⁸.

To determine the dependence of pA-U-G binding to ribosomes on fMet-tRNA, a mixture of 5'-³²P-labelled pA-U-G (100 pmol) and Q β [³H]RNA (2 pmol) was incubated with ribosomes (10 pmol) in the presence or absence of fMet-tRNA and 70S complex formation monitored by sucrose gradient centrifugation. As shown in Fig. 2, the binding of Q β RNA as well as of the pA-U-G was strongly dependent on the addition of fMet-tRNA to the incubation mixture: 0.3 pmol of Q β RNA (15% of input) and 0.6 pmol of pA-U-G (0.6% of input) were bound when initiator tRNA was added, and 0.06 pmol were bound in either case when the tRNA was omitted. The binding of pA-U-G was almost proportional to its input level, up to 100 pmol. At 10 pmol, the binding of pA-U-G was 0.08 pmol, about one-quarter that of Q β RNA at an input of 2 pmol. These results may indicate a different rate of complex formation for Q β RNA and pA-U-G; it is less likely that the yield of 70S complex would reflect an equilibrium between free and complexed components in the conditions of our assay, as centrifugation through a sucrose density gradient removes the excess oligonucleotide from the equilibrium. Oligonucleotide P-2(a+b) (a mixture of P-2a and P-2b) inhibited binding of Q β RNA but distinctly stimulated that of pA-U-G (Fig. 2c, Fig. 3) at all levels tested; the values for 40 pmol of P-2(a+b) were 78% inhibition for Q β RNA and 42% stimulation for pA-U-G. The corresponding values for 40 pmol of oligonucleotide P-6 (see Table 1) were 21% and 25%, respectively. The stimulation of binding of pA-U-G by P-2(a+b) may be due to increased availability of ribosomes no longer bound to Q β RNA.

There are great differences in the stabilities calculated for the putative interactions between the 3'-terminal region of 16S

rRNA and the Shine-Dalgarno regions of different mRNAs². It has been proposed that both the Shine-Dalgarno interaction and the interaction between initiator site and fMet-tRNA anticodon have a role in the formation and/or stability of the initiation complex^{2,5} and that one or the other may make the greater contribution, depending on the nature of the mRNA. Several sets of experimental findings bear on this hypothesis. (1) In the case of the Q β coat cistron, where the Shine-Dalgarno interaction is relatively weak ($\Delta G = -7.9$ kcal mol⁻¹), a mutation converting the A-U-G initiator triplet to A-U-A reduces the amount of 70S complex recovered in binding experiments to less than 10% (ref. 5). An A-U-G $\rightarrow$ A-C-G mutation in the cistron for the 0.3 protein of phage T7 (ΔG of Shine-Dalgarno interaction = -10 kcal mol⁻¹) does not diminish the efficiency of ribosome binding (in this case, however, it is not excluded that binding might occur at an A-U-G triplet two codons downstream)⁹. (2) In the presence of fMet-tRNA, *Escherichia coli* ribosomes bind to the coat cistron and to a lesser extent to the replicase cistron, but in its absence only to non-initiator sites which show extensive complementarity to the 3'-terminal region of 16S rRNA ($\Delta G = -11.1$ to -16.9 kcal mol⁻¹). Ribosomes bind efficiently, even in the absence of fMet-tRNA, to an RNA fragment from the R17 initiator region of the A cistron, where the Shine-Dalgarno interaction has a ΔG of -15.8 kcal mol⁻¹ (refs 2, 10). (3) The trinucleotide pA-U-G binds to 70S ribosomes, and at least one mRNA, the λ Prm RNA, which carries the initiation triplet at its 5' end and thus lacks the 'Shine-Dalgarno' region, is translated *in vivo*¹¹; however, binding of the trinucleotide pA-U-G seems to be far weaker than binding of Q β RNA as judged by the molar amount of the material retained by ribosomes, and the efficiency of translation of the repressor gene by Prm promoter is an order of magnitude less than that of Pre, the same mRNA preceded by a leader sequence comprising a Shine-Dalgarno region¹². (4) A point mutation diminishing the extent of the Shine-Dalgarno interaction at the initiator region of the 0.3 protein of phage T7 causes a shift of

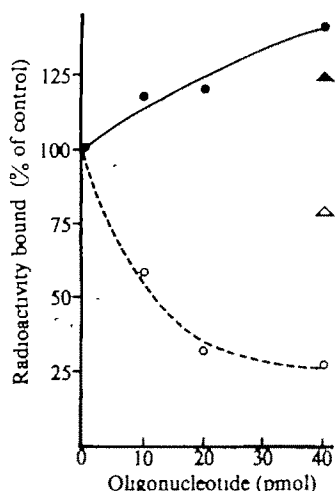


Fig. 3 Effect of oligonucleotide P-2(a+b) and P-6 on the binding of 70S ribosomes to Q β [^{3} H]RNA and [^{32} P]pA-U-G. Q β [^{3} H]RNA and [^{32} P]pA-U-G were incubated with ribosomes, fMet-tRNA and the amounts of P-2(a+b) or P-6 indicated, and 70S complex formation was determined as described in the legend to Fig. 2. $\circ$, Q β [^{3} H]RNA and P-2(a+b); $\bullet$, [^{32} P]pA-U-G and P-2(a+b); Δ , Q β [^{3} H]RNA and P-6; $\blacktriangle$, [^{32} P]pA-U-G and P-6.

ribosome binding to an A-U-G located 15 nucleotides downstream, whereas a suppressor mutation, which creates a more stable Shine-Dalgarno interaction at the original site, restores the normal ribosome binding capacity of the mRNA.

If P-2a blocks the formation of Shine-Dalgarno interactions, then ribosomes could only attach to Q β RNA by virtue of the interaction between fMet-tRNA and A-U-G. If we assume that there are no other important interactions between ribosomes and natural mRNA, the rate of formation or stability of complexes between 70S ribosomes and the trinucleotide A-U-G on the one hand and any other mRNA on the other should become similar in the presence of oligonucleotide P-2a. It will be of interest to compare the effect of this oligonucleotide on ribosome binding and protein initiation, both with Q β RNA and with other messengers.

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An intervening sequence of the mouse β -globin major gene shares extensive homology only with β -globin genes

THE mouse β -globin major (β^{maj}) gene is interrupted by two intervening sequences of DNA that divide it into three discontinuous segments¹⁻³. The entire gene, including the coding, intervening and untranslated regions, is transcribed into a colinear 15S mRNA precursor⁴ containing a 5'-cap structure and 3'-poly(A) (refs 5-7). Because mature globin mRNA is significantly smaller (10S) and does not contain these intervening sequences, the 15S precursor must be processed. Such processing presumably accounts at least in part for the reduction in sequence length observed between so-called heterogeneous nuclear RNA (Hn RNA) and cytoplasmic RNA⁸⁻¹⁰. Intervening sequences seem to occur in a variety of genes and organisms^{1,11-18}, but their function and representation in the genome have been unknown.

The availability of cloned β -globin genes^{3,16} permits us to answer certain obvious questions about the structure and occurrence of intervening sequences. Previously, we examined the extent of the homology between cloned β -major and β -minor (β^{min}) genes by means of restriction endonuclease analysis and by visualisation of heteroduplex structures formed between them. Of the 7,000 base pairs compared, shared non-coding sequences comprised only a few hundred base pairs¹⁶. In the longer (646 base pairs) intervening sequence, homology was found only in the regions bordering the structural gene sequences; thus, the central portion of the intervening sequences has diverged. The significance of this conserved homology, perhaps representing retained regulatory signals or sites important for the elimination of intervening sequences, is unknown. Any homology to other portions of the genome has likewise been unknown. Do parts of the intervening sequence constitute a genetic signal retained among unrelated genes throughout the genome, analogous to bacterial insertion



Fig. 1 Derivation of intervening sequence containing probes from β^{maj} (M β G2) DNA. *a*, The M β G2 mouse genomic EcoRI fragment containing the β^{maj} gene, including flanking, transcribed but non-translated, intervening and coding sequences. This fragment was cloned intact into the plasmid PMB9, yielding PMB9· β G2 (ref. 2). *b*, (IVS-A), PMB9· β G2 DNA was digested with *Bam*HI and *Hind*III (New England Biolabs), ligated with similarly digested PBR322 DNA, and used to transform χ 1776 (ref. 2). Clones hybridising the excised 230-base pair fragment were picked, grown up, and the 230-base pair *Bam*-*Hind*III fragment cut out of a 9% polyacrylamide gel, eluted by electroelysis and concentrated on DEAE cellulose. *c*, (IVS-B), PMB9· β G2 DNA was digested with *Bam*HI and *Pst*I, and the appropriate 620-base pair fragment isolated from a 9% gel as above. *d*, (IVS-C), PMB9· β G2 DNA was cleaved with *Bam*HI and *Xba*I and the 1,500-base pair fragment isolated as above except from a 5% gel. $\square$, Genomic flanking sequences; $\blacksquare$, coding sequences; ▨ , intervening sequences; ▤ , transcribed, untranslated sequences.

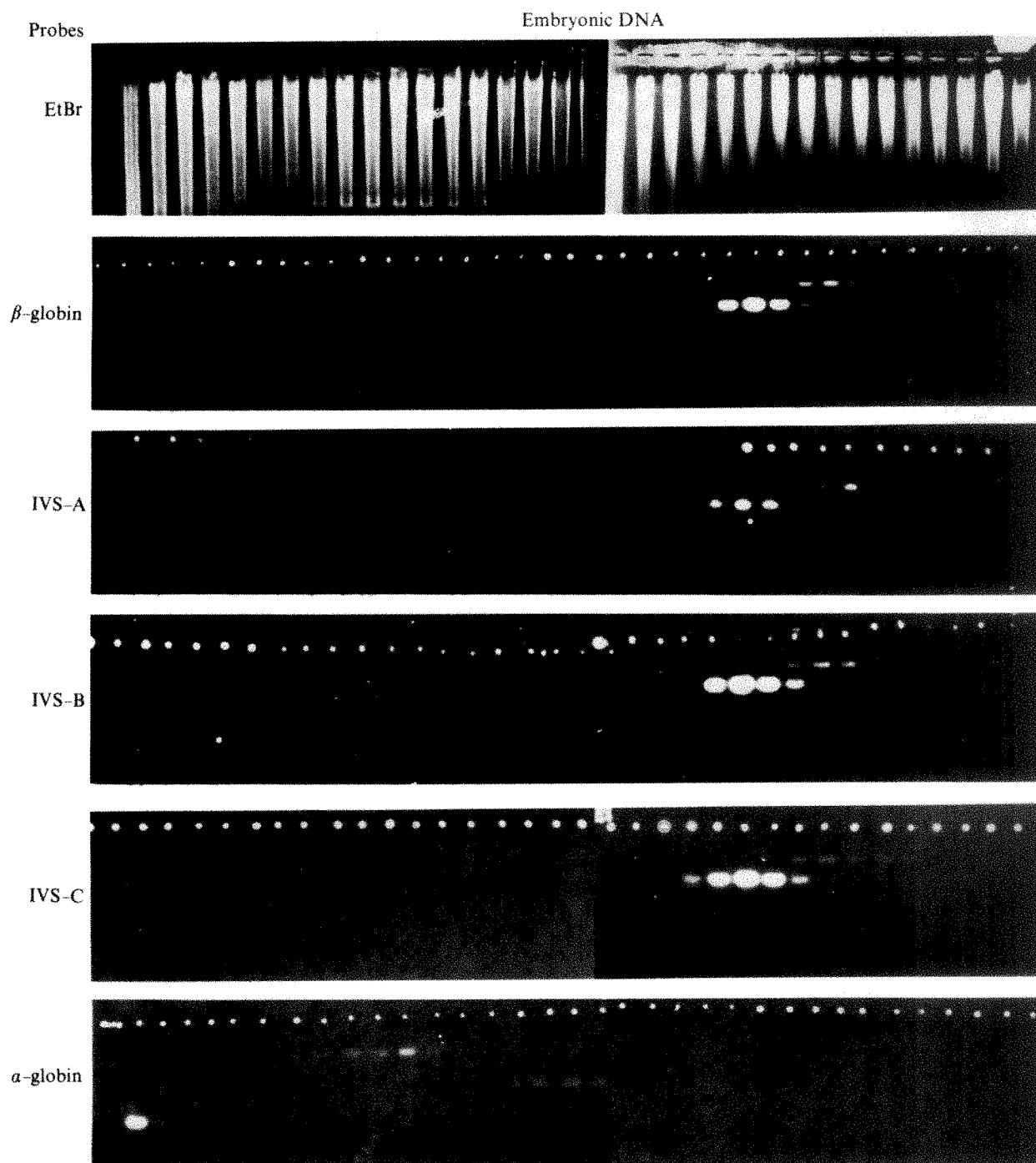


Fig. 2 Hybridisation to genomic DNA of various probes containing intervening sequence from β^{maj} (MBG2). EtBr, an ethidium bromide-stained 1% Agarose gel containing 35 pooled fractions from an RPC-5 chromatograph of *Eco*RI DNA; direction of migration is from top to bottom. The DNA in gels identical to the stained gel shown was transferred to a Millipore nitrocellulose filter¹³ and hybridised to various ^{32}P -labelled probes (see Fig. 1) as follows: ^{32}P -pCR1 $\cdot$ βM9 (β -globin); ^{32}P -IVS-A; ^{32}P -IVS-B; ^{32}P -IVS-C; ^{32}P -pCR1 $\cdot$ αM10 (α -globin). Probes were labelled by nick-translation²² to specific activities of 86 c.p.m., 158, 12.7, 143 and 18 c.p.m. pg^{-1} , respectively.

sequences¹⁹? Are intervening sequences shared among genes expressed coordinately in synthetic pathways, such as those involved in erythropoiesis? Or are intervening sequences completely unique? To answer these questions, we have used an especially powerful form of analysis involving a two-dimensional distribution of *Eco*RI fragments of mouse genomic DNA, RPC-5 chromatography and Agarose gel electrophoresis. The resulting pattern is then transferred to nitrocellulose filters and hybridised *in situ* to ^{32}P -labelled probes²⁰ containing all or part of the β^{maj} intervening sequence. We have examined the

genomic representation of the larger intervening sequence of the mouse β^{maj} gene and report here that it shares extensive homology only with elements of the β^{maj} and β^{min} genes.

Figure 1 shows the original cloned mouse globin gene, β^{maj} (MBG2) (Fig. 1a) and the probes derived from it. The β^{maj} gene is comprised of genomic flanking, coding and intervening sequences, as shown. Preliminary sequencing studies (D.A.K., unpublished) indicate that the 5' terminus of the small intervening sequence begins after the codon for amino acid 30; its precise length has not yet been determined. The first probe, derived

from a sub-clone of M β G2, is a 230-base pair fragment which extends from a *Bam*HI site at amino acid codon 99 to a *Hind*III site near the middle of the intervening sequence; this probe (designated IVS-A) contains the most distal 18 base pairs of the 5' coding sequence and the proximal 220 base pairs of the intervening sequence. The second probe depicted in Fig. 1, a *Bam*HI to *Pst*I fragment of approximately 600 base pairs (designated IVS-B), also contains the distal 18 base pairs of the 5' coding sequence but extends through the intervening sequence to within 75 base pairs of the 3' coding sequence. The third probe, an approximately 1,500-base pair *Bam*HI to *Xba*I fragment (designated IVS-C) contains in addition to the distal 18 base pairs of the 5' coding sequence, the entire intervening sequence, 3' coding sequence, 3' transcribed but untranslated region, and approximately 600 base pairs of genomic flanking sequence.

To identify sequences in the mouse genome homologous to these probes, murine embryonic DNA was purified, fragmented with *Eco*RI and fractionated by RPC-5 column chromatography. Aliquots from the RPC-5 fractions were electrophoresed, blotted on to nitrocellulose paper and then annealed to a ³²P-labelled probe^{16,20}. The results of hybridisations with the three probes are shown in the third (IVS-A), fourth (IVS-B) and fifth (IVS-C) panels of Fig. 2. The identical appearance of these three panels is notable, as is their identity with the second panel (β -globin), where the probe was the ³²P-labelled β -globin structural gene sequence cloned in plasmid pCR1 (ref. 21). The two discrete bands observed with the β -globin structural sequence as probe (and hence, those bands in the same positions in the IVS-A, IVS-B and IVS-C hybridisations) represent the two non-allelic β -globin genes: the upper, less intense band is the 13-kilobase β^{maj} (M β G3); the lower, more intense band, the 7-kilobase β^{min} (M β G2). The last panel (α -globin) demonstrates the band pattern of mouse α -globin sequences; in this experiment, the probe was the ³²P-labelled α -globin structural gene sequence cloned in pCR1 (ref. 21). Note that three α -globin genes are detected and that there is no homology between any of these and the β^{min} probes (compare IVS-A, B and C to α -globin). The top panel (EtBr) does not depict an autoradiogram but is simply an example of a gel stained with ethidium bromide and photographed before blotting and hybridisation; note the large amounts of DNA of heterogeneous size in each RPC-5 fraction, each lane containing of the order of 10⁴ genomic fragments.

Before drawing conclusions from the above experiments, we should consider the ability of our probes to detect homologous sequences. From a comparison of the partial sequences of M β G2 (β^{min}) and M β G3 (β^{maj}) as well as heteroduplex studies, we are certain of at least 50 base pairs of homology between probes IVS-A or IVS-B and β^{min} (ref. 16; D.A.K., unpublished data); it is evident from Fig. 2 that this degree of hybridisation is detected. We know, however, from unrelated experiments, that IVS-A barely detects an 18-base pair homologous sequence (H.I.M., unpublished data). The minimum size of homologous sequences resolved in these experiments is, therefore, approximately 20–50 base pairs.

We conclude that the long intervening sequence of the mouse β^{maj} gene shares detectable homology in the mouse genome with only the β^{min} gene. We cannot rule out short sequences which might be more widely shared among intervening sequences and serve as recognition sites for processing. It is possible that rather than similarity or identity of primary sequence, there are within the intervening sequences characteristic conformations conferred on the mRNA precursors by secondary and tertiary structures. Alternatively, the sequences surrounding the intervening sequence may provide the specificity required for the elimination reaction. Such a possibility is especially suggested by what is known of yeast tRNA intervening sequences^{12,17}. These differ among tRNA precursors, whereas the conformations of the tRNA molecules themselves are probably quite similar. Further, recent evidence involving a portion of a putative intervening sequence in the chicken ovalbumin gene suggests that it is

present only in the ovalbumin gene¹⁸. Ultimately, a comparison of the structure of all these elements should permit us to define the critical features required for splicing.

Note added in proof: We have recently determined the entire nucleotide sequence of the mouse β -globin major gene (Konkel *et al.* *Cell*, in the press) and find short closely homologous sequences at the borders of both small and large intervening sequences.

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Errata

In the article 'The tau heavy lepton—a recently discovered elementary particle' by M. L. Perl, *Nature* 275, 273–278, in line 6 on page 274 for reaction (2) read reaction (3). In line 13 for equation (1) read equation (2).

In the letter 'Viral sequences related to a human skin papillomavirus in genital warts' by G. Orth *et al.*, *Nature* 275, 334–336, on page 335 right-hand column line 6 should read ... are close to those ...

The title to the letter by Jones, Kindman and Knowles, *Nature* 275, 564, should read: 'Stereochemistry of phosphoryl group transfer using a chiral [¹⁶O, ¹⁷O, ¹⁸O] phosphate monoester: the stereochemical course of alkaline phosphatase'.

matters arising

Possible Sun-weather correlation

GERETY ET AL.¹ recently repeated part of my work² on the correlation between solar activity and weather for the zones 50°N–60°N and 60°N–70°N. I was not surprised by the fact that the authors have found substantial differences between my results and theirs for the simple reason that the two statistical samples on which the papers were based differ significantly. In fact, in my work I have used only those stations which provided long-term observational data, whereas Gerety *et al.* used stations with observational data even of 1 or 2 yr. Furthermore, in my paper I excluded a few annual values of $R - R_0$ which appeared to differ significantly from the mean annual value. However, despite these remarkable differences between the two statistical samples, Gerety *et al.* have found a general similarity in the variation of the precipitation in the two northern zones. This can be clearly seen in Fig. 1 where the continuous lines represent my calculations and the dashed lines the calculations of Gerety *et al.* One could not expect to obtain a closer correlation from a comparison of two statistical samples so different in the kind and the number of stations used in the cited papers.

JOHN XANTHAKIS

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1. Gerety, E. J., Olson, R. H. & Roberts, W. D. *Nature* **272**, 231–232 (1978).
2. Xanthakis, J. *Solar Activity and a Global Survey of Precipitation* (Research Center for Astronomy, Academy of Athens, 1975).

GERETY REPLIES—Although in preliminary analyses stations with only a few years data were included, the study published in *Nature* included no stations with a length of record of < 15 yr. To be more specific about the statistical similarity between our separate samples: (1) In the latitude band 60°N to 70°N Xanthakis used 42 stations. Three of these stations had record lengths of less than 15 yr and two were located in another latitude band entirely. Our sample included all of those used by Xanthakis with the exception of the five just discussed and two more for which we could find no data. The total number of stations in our sample was 97. (2) In the latitude band 50°N to 60°N Xanthakis used 71 stations, one of which had a record length of less than 15 yr. Our sample included all but five of the remaining stations. The total number of stations in our sample was 159.

We agree with Xanthakis that there is a “general” similarity between his curves

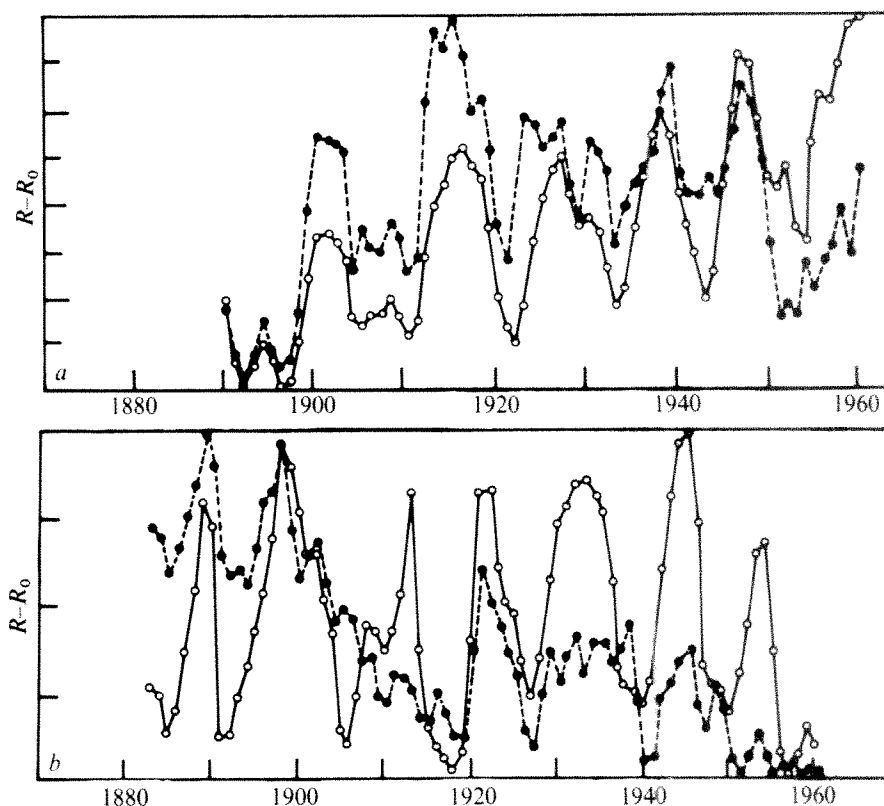


Fig. 1 Comparison of the calculations of Gerety *et al.* (dashed line) and the author (continuous line).

and ours but fail to see any hint of correlation between our curves and solar activity as measured by Xanthakis' index Ia. Finally, if there were in fact a correlation between Ia and precipitation measured in this peculiar manner, it seems to us that it ought not to depend so strongly on one's choice of stations.

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Oxygen in the palaeoaquatic environment

The article by B. and A. Henderson-Sellers has an inappropriate title because it is not concerned with oxygen in the palaeoaquatic environment *per se* but attempts to identify, at the end of the Precambrian, the most likely region on the Earth where diversification of multicellular animals took place, by examining oceanic oxygen levels from the average

position of the density gradient or pycnocline at that time. Thus they show pycnocline depth as a function of latitude, 600 Myr ago, on the basis of a model which depends on several assumptions, the principal being that the palaeocean was density-stratified like the water of a temperate lake during the summer², and from an assessment of the concentration of oxygen above the pycnocline (acting as a barrier to downwards diffusion of oxygen released by photosynthetic organisms) they conclude that multicellular animals originated, and diversified, in latitudinal zones between ~50 and 70° N and S. Their decision to examine the distribution of oceanic oxygen levels 600 Myr ago was evidently influenced by the very late and sudden diversification of life, that “apparently” took place at the end of the Precambrian.

Assuming that 600 Myr ago oxygen was limited to water above the pycnocline, the Henderson-Sellers have suggested only that, at that time, the surface waters of certain latitudinal zones contained a sufficiently high concentration of oxygen to be suitable for the existence of multicellular life. They have not provided evidence showing that such conditions did not exist at the same or different latitudes before 600 Myr ago and, therefore, have

failed to tie the origin and initial diversification of multicellular animals either to the end of the Precambrian, or to particular latitudinal zones. Evolutionary diversification must have been taking place at that time because once initiated, it is continuous. Eucaryotic unicellular organisms dependent on oxygen were living at least 300–400 Myr before the beginning of the Cambrian³, and the fully aerobic environment in which they existed⁴ was equally suitable for multicellular animals. That this was the situation for at least 100 Myr before the end of the Precambrian is shown by the presence of fossils of a variety of soft-bodied metazoans in late-Precambrian rocks in several parts of the world^{5,6}. These late-Precambrian organisms were, however, already complex forms, so that the evolution of metazoans from protists⁴ clearly took place still earlier in the Precambrian, and was not connected in any way with environmental conditions at the end of the Precambrian.

The concept of a sudden diversification of life at the end of the Precambrian derives from the time when it was widely believed (from the lack of fossil evidence) that metazoans evolved only shortly before the beginning of the Cambrian. Thus, Cambrian strata, in contrast to apparently unfossiliferous Precambrian rocks, were known to contain abundant fossils representing the principal animal phyla now inhabiting the world as well as extinct ones. The abundance of fossils at that time was a result of the acquisition of preservable hard parts independently by many groups of organisms. Although the latter was not synchronous, it seemed more nearly so when the Lower Cambrian was believed to represent a period of 30 Myr as against the currently recognised 50 Myr (ref. 7). The complex multicellular organisms that developed hard parts were, however, not living in the surface water of the ocean but on the sea floor, and so they were derived from less complex forms already adapted for a benthic existence. Hence, the sudden diversification of life attributed to the end of the Precambrian or to early Cambrian time refers only to the bottom-dwellers that acquired skeletons, as little is known about the variety of soft-bodied benthic and pelagic animals also living at that time.

Restriction of oxygen, before the beginning of the Cambrian, to surface or near surface water⁸ (above or within the pycnocline) or, in high concentration, to certain latitudinal zones, implies lack of disturbance of the density layers by currents like those of today which move water from one region to another or from one depth to another, and result in a mixing of water and distribution of oxygen to nearly all parts of the ocean. An ocean in which movement of water bodies was too slow to significantly affect the stratification would require a more uniform heat distribution than there is at present; such conditions

could only exist in the absence of continents (differential heating giving rise to complex wind patterns, movement of water bodies, and so on) or even of significant rises on the sea floor, that is, the ocean would have to have been global and of more or less uniform depth⁹. Emergence of continents took place long before the end of the Precambrian^{10,11}, and oceanic conditions would then have been comparable to those existing today. During the Lower Cambrian the world consisted of at least five continents separated by oceans^{12,13}, and in the latter, multicellular animals existed at both high and low latitudes¹². Colonisation of the sea floor and overlying waters took place more than 100 Myr before the beginning of the Cambrian as the oldest known metazoan faunas include both benthic and pelagic forms^{6,15}; some of them lived in shallow water whereas others inhabited relatively deep water⁶. Consequently, as far as the fossil record of Precambrian multicellular animals goes back, oxygen was present in water well below the level of the pycnocline.

If multicellular animals, from the time of their origin until 600 Myr ago, had been restricted to the surface water of certain latitudinal zones because oxygen was not available in sufficient concentration elsewhere in the ocean¹, either near the surface or below the level of the pycnocline, diversification would have been inhibited, as their migration (actively, or passively by currents) into other environments where they could survive and where adaptive evolution could take place would have been impossible.

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Matters Arising

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- 5 Gleason, M. F. *Geol. Soc. Am. Bull.* **82**, 509–512 (1971)
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B. AND A. HENDERSON-SELLERS
REPLY—Many of Anderson's points are outside the scope of our paper¹, as the temporal evolution from single- to multicellular life within the evolving ocean-atmosphere system is a wide topic and is discussed in more detail elsewhere². The choice of 600 Myr ago as an example of our model results² was intended to illuminate the discussions of evolutionary processes current in the literature. Many of his points are, of course, still widely contested. However, we attempt to answer the most obvious below. The question of oceanic stratification at our chosen period is still unresolved. Degens and Stoffers³ discuss the anoxic conditions at depth resulting from the chemistries associated with the two layers. Although the permanency of this stratification is in doubt, its certain occurrence lends credence to our model and provides at worst error bars on the timing. Hargraves⁴ postulation of a global sea, although recently criticised⁵, seems to be supported by more recent evidence and discussion^{6,7}.

The existence of a pycnocline, at least for long periods during the ocean's history, implies that the build up of oxygen in the upper layers did occur at times up to and including the diversification and emergence of land animals. Our article was intended to illustrate an additional and important factor in the total description of the complicated process of global evolution. The dynamic feedbacks between life, ocean and atmosphere during the evolution of the Earth will not be simply resolved.

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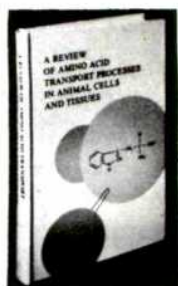
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reviews

Newton's correspondence

I. Bernard Cohen

The Correspondence of Isaac Newton. Vol. VII: 1718–1727. Edited by A. Rupert Hall and Laura Tilling. Pp. 522. (Cambridge University Press: Cambridge and London, 1978.) £25.

WITH this seventh volume, the Royal Society's edition of the *Correspondence of Isaac Newton*, begun under the editorship of H. W. Turnbull (vols 1–3) and continued under J. F. Scott (vol. 4), is now brought to a conclusion. Volume 7 (like vols 5–6) has been edited by A. Rupert Hall and Laura Tilling. The editors observe that very few of the letters included here "may justly be described as scientific". There are exchanges with Varignon and Bernoulli about the calculus, but not its subject matter so much as a rehearsal of the old controversy with Leibniz of a decade earlier over questions of priority in invention. Similarly, an extended set of correspondence with Varignon concerning the *Opticks* deals not with Newton's experiments and theoretical explanations, but rather with the type fount, the details of printing, the illustrations, and so on. There are letters from Pemberton to Newton about the third edition of the *Principia* (1727), which Pemberton was seeing through the press. But Newton's replies are lost and, in any event, most of Pemberton's queries to Newton do not concern fundamentals of mathematics or of physics.

Perhaps the most interesting letters in this volume are those written by or to Pierre Varignon, then a professor of mathematics at the Collège Mazarin and an influential member of the Royal Academy of Sciences in Paris. Varignon was considered by both Leibniz and Johann Bernoulli to be a partisan of the Continental mathematicians, but he "maintained at least a superficial impartiality in the debate", and kept up a friendly contact with both Newton himself and Abraham De Moivre, one of Newton's chief spokesmen. Today's reader may still be astonished by both the heat of the debate and the degree of chicanery and plain dishonesty on the part of Newton, Leibniz, and practically everyone who was in any way associated with the issue. For example, Newton told Bernoulli that he had not "ever taken the trouble of spreading opinions

throughout the world"; when he had actually written the draft of the report of the Royal Society's committee to examine the question of the true discoverer or first inventor of the calculus. Nor was that all; on the publication of the committee's documentary report in favour of Newton, the *Commercium Epistolicum*, Newton wrote and published a lengthy (anonymous) summary or 'review' of it in English in the Royal Society's *Philosophical Transactions*. This was translated into Latin and published in a reprint of the *Commercium Epistolicum* with an additional preface (also anonymous) by Newton. As to Bernoulli, he said of a famous letter he had written (also published anonymously), "I am not certain of what kind that letter addressed to Mr Leibniz is of which you speak . . . I do not remember having written to him myself that day, yet I would not deny it altogether . . .".

On a different level altogether is a brief note to Fontenelle, in relation to the presentation to the Royal Academy of Sciences of the second Latin edition of the *Opticks* (1719). Referring to the novelties introduced in this edition, notably the additional Queries, Newton said that "Here I cultivate the experimental philosophy as that which is worthy to be called philosophy" and he added that in his treatise "I consider hypothetical philosophy not as knowledge but by means of queries".

The volume concludes with a discussion of Newton's genealogy and a lengthy appendix, containing additions and corrections to the first six volumes. A considerable part of these addenda deal with Newton's activities as Warden, then Master, of the Mint. It is thus seen that Newton's post "was clearly no sinecure". Not only did he have the difficult job of supervising the construction and operation of County Mints to "receive and recoin the old hammered money", but he took it on himself to attend to the interrogation and prosecution of counterfeiters. These new letters deal with the chronology of Newton's discoveries in the calculus, chemical experiments made by Boyle, and a draft of a proposition for the revised *Principia* (1713) dealing with the resistance experienced by a cylinder that moves through a fluid. In a letter of January 1675, Newton

thanks the Secretary of the Royal Society for the offer of remission of annual dues, explaining that he could no longer afford to remain a Fellow, as "the time draws near that I am to part with my Fellowship, & as my incomes contract." Newton evidently was to resign from his Fellowship at Trinity College in spring 1675, as he could not in conscience go into Orders, as was then required of all Fellows; in April 1675 the Lucasian Professorship—to which he was appointed—was exempted by Royal Patent from the rule about Orders, but for which Newton's professional career would have been severely interrupted.

Scientists and historians and philosophers of science will be grateful to A. Rupert Hall and Laura Tilling on having at last brought to completion the edition of Newton's correspondence, of which the first volume was published in 1959. But any users of this set will regret that the last volume does not contain a comprehensive index to the complete correspondence. Furthermore, the corrections and emendations at the end of vol. 7 apply to all previous volumes, but they neither incorporate the previous list of errata (at the end of vol. 3), nor do they refer the reader to the entries in the earlier list. Thus, every time a letter in vols 1–3 is consulted, the reader must look in two separate lists of errata, which is a great inconvenience. □

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Thin-layer chromatography

Practice of Thin-Layer Chromatography. By J. C. Touchstone and M. F. Dobbins. Pp. 381. (Wiley-Interscience: New York and Chichester, UK, 1978.) \$27.30; £14.50.

PAPER CHROMATOGRAPHY was taken up slowly and with some fear and trepidation by those brave enough to try it at all because neither the paper nor the solvents and reagents were suitable for the technique; and, indeed, it took some time for paper for chromatography to appear on the market. Conversely, thin layer chromatography

took off explosively because, by the time it appeared, most of the general problems of the technique had been solved and it was a relatively simple matter to transfer from paper to thin layers.

If you are about to start chromatography then this book will not describe how to separate the compounds of interest, be they aminoacids, steroids, dyes, and so on. However, if you have some or even a great deal of experience then it is a mine of information covering the whole subject in a most up-to-date manner. Extensive discussions on preparing plates, pre-coated plates, the mobile phase, types of chromatography, documentation, quantification and reproducibility, preparative and other aspects of the techniques are given in a very readable

manner. Many tricks of the trade are described and I found some which I had not picked up in over twenty years of experience. Conversely, I feel that I might add a few to their repertoire. The best way to cut foil-backed layers is with a guillotine and to prevent glass capillaries scratching plates it is best to place 1-2 mm plastic tubing over the end which will be applied to the plate.

This book can be confidently recommended to all who practise thin layer chromatography and it would be a valuable addition to all departmental libraries.

Ivor Smith

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Animal communication

How Animals Communicate. Edited by T. A. Sebeok. Pp.1128. (Indiana University Press: Bloomington and London, 1978.) £40.

ABOUT ten years ago, T. A. Sebeok edited two books on animal communication, one of which became a standard reference work in the field. The present volume is intended to update and replace both of these earlier ones. Fitting two into one and taking account of progress has led to a massive volume at an astronomical price.

Editing a book to which there are 38 separate contributions must be a daunting task if the contributions are to appear on time and are to be well integrated with each other. Unfortunately, Sebeok has cut corners in these respects, the different chapters varying greatly in style, organisation, degree of detail and length, and many of them (presumably those by the more punctual authors) containing no references after 1974. Strictly imposed word limits tailored to the importance of each author's topic and more rigorous selection of the topics to be covered would have made a shorter, better and cheaper book. This is not to say that the book does not deserve some applause: many of the individual chapters will be, within their limited areas, the definitive reviews and works of reference which the whole book was intended to be.

One reason for its length is that the book is split into three sections, each classifying the field in a different way and thus involving some overlap. The variability of treatment is well illustrated by the first section, which deals with theoretical issues. Here there are three thorough and useful chapters on

evolution (Marler), ontogeny (Burgardt) and action patterns (Barlow). In the other three Lieberman repeats his controversial ideas on the evolution of language, Griffin gives a brief personal perspective and Robertson reviews cellular communication, a topic of little relevance to the book's main theme. The second section deals with communication split up according to the senses and is equally patchy. There are useful conventional reviews of pheromones (Shorey) and visual signals (Hailman), as well as two excellent ones on more specialist but intriguing phenomena: bioluminescence (Lloyd) and electric signalling (Hopkins). Beside these are a more or less irrelevant article on echolocation (Griffin), an out-of-date one on acoustics (Busnel),

reprinted from one of Sebeok's earlier books, and a chapter on tactile communication which deals only with humans (Geldard).

The third section of the book, which accounts for three-quarters of its length, covers communication in selected animal groups in a series of 25 chapters. Some of the groups about which less is known are dealt with almost species by species, yet a more restricted approach has had to be adopted to those which have been more widely studied. Hölldobler shows how well this can be done with his article on social hymenoptera, and the artiodactyls (Walther) and higher primates (Oppenheimer; Gautier and Gautier; Marler and Tenaza) also receive good thorough treatment. The birds lose out a bit with a relatively brief and general article by W. J. Smith. The book ends with an article by Sebeok on the relevance of animal communication to human studies, preceded by one on man-chimpanzee communication by Fouts and Rigby. The editor's view on the rather sterile controversy as to whether the feats of chimpanzees constitute language is neatly expressed by the appearance of the names of these chimps in what would otherwise be an author index.

Overall, this book contains much that is readable and interesting, but it falls short of being an encyclopaedic work of reference because it covers some areas very much more fully than others.

P. J. B. Slater

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Interpreting fossil assemblages

The Ecology of Fossils: An Illustrated Guide. Edited by W. S. McKerrow. Pp.384. (Duckworth: London; MIT Press: Cambridge, Massachusetts, 1978.) £14; \$22.50.

THIS book is the first attempt to portray fossil communities of marine invertebrates from every geological system since the Precambrian. It contains 125 block-diagrams of fossil assemblages, each picturing animals and/or plants in life-position, in many cases their position when buried as fossils and their appearance in cross-section in the sediment. Each assemblage is described and for each system there is a general introduction. The book owes a great deal to Mrs. E. Winson for her excellent drawings. The fact that different taxa may be impossible to distinguish in these diagrams —

for example, *Orthambonites* and *Pleurorthis* in fig.5, or the two species of *Barillaria* in fig.115 — is a useful reminder to the student that fossils cannot usually be identified properly at a glance; but too many of the pictures are given impossibly accurate names.

Although biologists do not agree amongst themselves on how a community can be defined, the authors consider that there is only one way to define fossil communities: a group of animals living in the same habitat. This convenient empirical definition is elaborated in the introduction to the Silurian, and it becomes apparent that they recognise that it is the associations of taxa themselves that define both the habitat and the community living in it: habitat is not intended to be defined by the inorganic facies of the sediment. Nevertheless, many of the communities in this book are tied to particular facies environments — for example, "Upper Devonian Clastic Shelf" or "Muddy Lime Sand" (in the

Jurassic).

The authors recognise that the communities illustrated are a selection from the large number known and that the diagrams emphasise organisms that are common and easily preserved. The implication is still there that the assemblages illustrated are real associations of fossils. This is not always true, and it is noticeable that many of the "communities" are not tied to named stratigraphical units. Thus, fig.85, the Upper Jurassic "Calcarene Community", which from the presence of *Rhacella* can be presumed to be a Corallian assemblage, is unlike any single example of the 18 detailed benthic associations in the Corallian as described by Fürsich (*Palaeontology*, 20, 337-385; 1977). Similarly, the *Bostrychoceras* in fig.107 is unknown from the British Maastrichtian chalk "community", unfortunately here called the "Ostrea Lumata Chalk Community" after a species that is actually limited to a very small thickness of the succession that happens to lack many of the other fossils illustrated. These minor mistakes are only part of a general weakness, and perhaps inevitable limitation, of all the reconstructions: they are both unquantitative and subjective. Why include so few ammonites in the Jurassic where they are often abundant, and yet provide an illustration containing seven different species of ammonites in the Late Turoonian Hardground Community (fig.105), which in turn does not include or mention the much more common brachiopods? Why is a *Caninia* not illustrated in fig.50 when it is mentioned in the description of the community? Conversely, *Aviculopecten* is illustrated but we are told nothing of its ecology.

For a book on ecology, the actual amount of ecology given is often very limited, even allowing for the fact that many of the groups of organisms are extinct. For the Devonian, Permian, Triassic and Jurassic there are good attempts at an integrated interpretation of each community, but for many animals in other chapters nothing is given beyond whether it was benthic, planktic or nektonic. Quantitative information is scarce throughout. It is not explained that the linear scales to the diagrams are only to size the organisms, not to indicate the number of specimens that one can expect to find in the rock, nor their original abundance.

Although these weaknesses will be obvious to the professional, the book is likely to be welcomed by students. They will value the novelty of fossils being interpreted in assemblages that they might find for themselves, as opposed to a theoretical discussion of principles or a mere list of facts about

each phylum separately. Although mainly for the British reader, the associations are hardly tied to national frontiers. It would be equally useful elsewhere in northern Europe, and many of the Palaeozoic associations can be found in the eastern part of North America. Moreover, at least an outline is given in many chapters of modifications to be expected in North America and the Tethys. Future editions could be improved by the inclusion of a few famous foreign examples such as the Burgess Shale fauna and a rudist reef association; there could also be more examples of present-day communities, each with a fuller ecological synthesis. Most of all

the bibliography needs to be classified: at present it is no more than a list of the papers referred to. As a result there are three petrological papers by West, but on ecology only one each by Bromley, Fürsich and Kauffman and just two by Sellacher. No reference is made to work by Bulman, Carter, Bager, Hudson, Kirk, Nicol, Oliver, Sohl, Reid or Scott, just to mention some well known names who write in English. The only serious misprint is on p110 where none of the key letters to the diagram has been printed.

J. M. Hancock

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Communication update

On Human Communication: A Review, A Survey and a Criticism. Third edition. By C. Cherry. Pp.374. (MIT Press: Cambridge, Massachusetts, and London, 1978.) £12.25; \$21.85.

THE re-appearance of this book is both timely and welcome. Since the first edition was published the world has seen the emergence and explosive growth of information processing systems of all kinds — not only large-scale electronic computing machines, with associated developments in storage media, data-bases and information retrieval, but many others. Thus we have: satellite and ground-based microwave communication systems that have transformed the world into McLuhan's 'global village', allowing an estimated 0.7×10^9 people to view the late pope's funeral as it took place; new components for the optical transmission and manipulation of information, promising even greater speeds and data densities; private and commercial networks for linking many computers together; large on-line systems for airline reservations and hospital management; and, most recently, developments in component technology that have not only turned computers themselves into mere components, at component prices (so-called microprocessors), but have also allowed the economical production of a host of other 'intelligent' components that have revolutionised systems design in all areas of electronic information handling. Many such systems perform critical functions in society on the basis of information gathered by dialogue with people. By improving and disseminating knowledge of human communication we may hope to avoid such communication disasters as apparently occurred with the signal control system for Britain's M4 motorway in December

1976 when 11 people were killed or injured and the motorway was closed for over 6 hours.

The book is unchanged from the first edition, apart from the addition of one chapter and minor corrections. It is a tribute to the scope and fundamental nature of the book that it survives well this procrustean decision that would have proved fatal to books of lesser stature, but certain sections are consequently rather dated (for example, those touching on machine intelligence and perception, including the machine synthesis and recognition of human speech). Nearly all the shortcomings of the book arise from this source and from failure to update material prefaced by terms such as 'recent' and 'today'.

The book defines communication as an act of sharing, distinguishing sign systems of animals from human language, and symbols from words, noting that language is the basis of rational thought and conduct. It then traces the historical development of our current views on 'information', and goes on to examine and describe language. There is next a review of various methods of analysing speech; but by failing to treat modern developments the author omits mention of Linear Predictive Coding of the speech waveform as well as the Fast Fourier Transform, both of which are highly relevant to computer-based analyses of speech. Then the classical Wiener-Shannon statistical theory of communication is simply explained and the author goes on to show how Bar-Hillel extended notions of information measurement to the semantic domain, based on Carnap's theory of inductive probability. Chapter 7 reviews some of the basic facts and problems of human perception and cognitive activity, ending with the observation that it is the multitude of general concepts and powers of organising them by means of language that form the backbone of human communication, dis-

tinguishing humans from animals.

In the new chapter 8, explaining what is human about human communication, in the light of the recent interesting experiments on communication with apes, there is useful insight (for instance, the idea that classical stimulus-response psychology may be rejected as the sole basis for understanding humans, because such a restriction would rule out human communication and would fail to distinguish one's relationship to a person from one's relationship to a thing). The author also sets down an existentialist view of what it is to be human. However, in excluding computer 'languages' from the domain of true languages—and thus from human communication, preferring to call such constructs coding systems because they require no cognitive effort on the part of the machine — the author touches on an important point about human communication that is not followed up, despite being implied at numerous points throughout the book. The point is that we shall not truly understand human communication until we can understand what kind of cognitive effort is involved; until we can formalise just what changes occur in a receiver's world model, and know how to produce those changes most efficiently, or at least can state what kinds of signal strategies are most likely to be useful. A reference to work by Winograd, Winston, Charniak or Colby would seem relevant here.

The book is somewhat preoccupied with spoken and written symbolic forms of language, at the expense of graphical communication. It is still true that a picture is worth a thousand words, and some forms of dialogue are greatly facilitated by providing for much of the information to be communicated and manipulated in the graphical domain, as instanced by the work of Robert Spence, at Imperial College in London. Indeed, as far as communication between humans and machines is concerned, it is in this area that the greatest progress has been made, perhaps for the same reasons that pictures were used at an early stage in human-human communication. Pictorial representations can have just as rich a complex of syntactic, semantic and pragmatic rules, just as many paralinguistic features of various kinds, as written — if not spoken — forms of what is generally called language; but it seems probable that there is some fundamental difference in the way that picture or spatial reference information is handled by the human.

The general reader will undoubtedly wish to skip the mathematics, mainly in chapter 5, but will find the ground

has been carefully prepared for what follows at each stage of the developing argument. Great care has been taken with the choice of words and the form of expression, and the book is rich with insight and painstakingly collected background material. The historical references, the relevant asides, and the anecdotes are a pleasure and a real aid to understanding for all readers.

Pesticide ecology

Ecology of Pesticides. By A. W. A. Brown. Pp.525. (Wiley: New York and Chichester, UK, 1978.) £17.65.

THE title of this book seems highly relevant to the current interest in 'ecotoxicology'. The author is well-known and respected for his work on resistance of insects to insecticides, a topic that with its strong basis in genetics should lead easily into ecological considerations. In the event, I found the book a sad disappointment.

The first, introductory, chapter gives a brief outline of the main types of pesticide, the economic and medical reasons for their use, their potential hazards to man, and of resistance to insecticides. The bulk of the book, in thirteen chapters, takes insecticides, herbicides and fungicides in turn, and considers their effects on selected components of various habitats. For example, chapter 6 considers insecticides and fish, whereas chapter 8 considers insecticides and birds. This approach makes for a repetitive and superficial account of what could be unifying themes. Inhibition of cholinesterases by organophosphorus insecticides could provide one such theme. Instead, chapter 1 briefly states this as the mode of action, and chapters 6 and 8 give a few field observations on the degree of inhibition that is associated with death. There is little mention of the range of cholinesterases and acetylcholinesterases, and no mention of demyelination. There is no discussion of the possible implications for sublethal effects or of the difficulties in deducing from cholinesterase inhibition whether death is due to insecticide poisoning.

More important, there is a lack of ecology, which should surely mean a consideration of the interactions between pesticides and other factors, and their effects on population size and on community structure. Instead, a large part of these chapters consists of statements of the percentage reduction in population size for species exposed to pesticides in the field, of LD₅₀ and LC₅₀ values, and of residues found in

However, for the serious student of human communication, the book must be regarded as what it is in 1978 — only a beginning. Nevertheless, for pleasure or study, the book will prove a very sound investment.

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tissues. The amount of data is overwhelming, and seems more suitable for reference than for reading. This may reflect the author's prefatory statement that the book metamorphosed from a concise text into a detailed review. The relevance of this material to the understanding of ecological events is difficult to see: when one deliberately kills members of one species changes in other species are bound to occur, irrespective of any direct effects that the pesticide may have on these other species.

At least one ecological principle is invoked, that maximum species diversity gives greatest stability to an ecosystem. This is accepted in chapter 2 without question and without supporting evidence, whereas there are many who would argue the opposite.

Chapters 9 and 10 are devoted to more general themes. The first considers organochlorine insecticides and eggshell thinning. The second considers the movement of insecticide residues, locally and globally. Food chains are considered in some detail, and are shown to concentrate persistent organochlorine insecticides. The account is, however, somewhat partial. Important conflicting evidence is not quoted, and other evidence is not critically appraised. Thus, in one example, concentrations of DDT in predatory fish and birds are given for visceral fat, whereas the concentrations in plankton and small fish are for whole bodies.

There is something of a North American bias in the material considered, with some notable omissions in references to the literature, and readers from other continents may wish that latin names had been given for all species, as well as their common names. These comments aside, the subject index lists both individual pesticides and individual species effectively, and the book could be useful for quick reference to some information on the acute toxicity of pesticides, their persistence and breakdown products.

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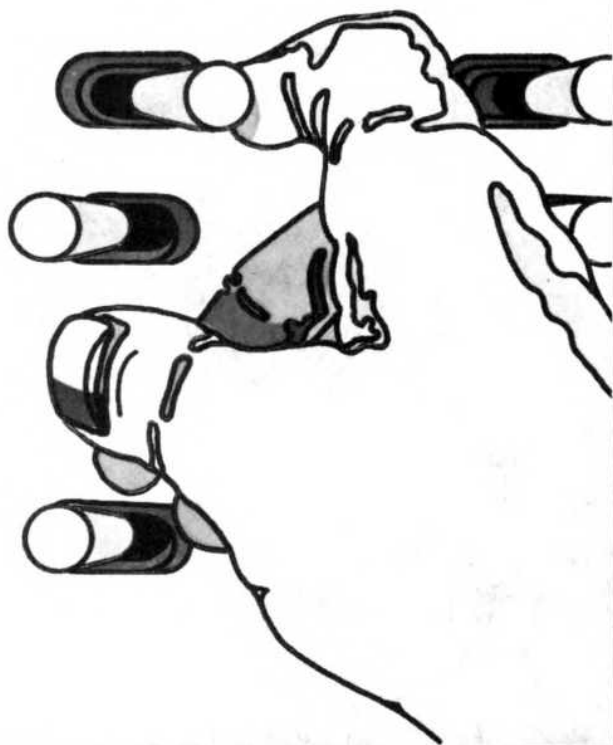
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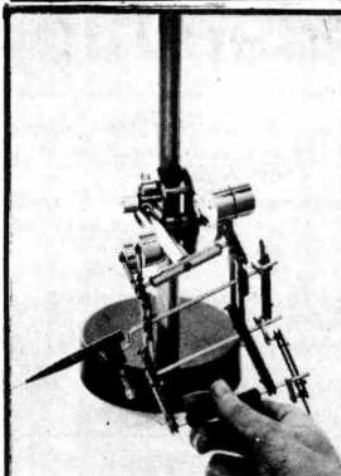
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CAMAC—system design made easy

Not so many years ago oscilloscopes were being designed and built by research scientists for use in their own experiments. Today such a situation is rare. Oscilloscopes have become standard items that are commercially available but the need for complex electronics to collect data and control experiments has increased. Many research groups are once again facing the problem of designing and building complex data processing systems.

The use of small on-line computers is not a complete solution. A computer is often necessary to handle the volume and rate of data but it must still be connected to the experiment. To make progress in research an experimenter must acquire considerable expertise in computer science and electronics engineering. The problem first became acute in nuclear physics, and a standardised solution was sought. Big science could afford to provide professional support teams to design and build interface electronics and the expertise and experience of these teams have ensured that the solution is tailored to the needs of the research scientist.

The solution is called CAMAC. It has been commercially produced for some years now, it works and is available internationally at a reasonable price. More and more areas of research are turning to computer based systems and the existence of CAMAC ensures that the link between the computer and the experiment can be implemented painlessly.

The following three articles, written by recognised experts in the field, introduce the CAMAC system. They illustrate what CAMAC is, why it is used and how it is used. We hope that this introduction will remove many of the problems often associated with the use of small computers and will ensure that progress in research will be able to take full advantage of progress in electronics.

CAMAC specifications are now available as international and national standards. Further information and details of how to obtain documentation can be obtained from the following:

L. COSTRELL
National Bureau of Standards,
Washington, D.C. 20234

H. MEYER
ESONE/ECA Secretariat
Commission des Communautés Européennes,
CCR-BCMN, B-2440 Geel, Belgium

Introduction to the interface standards

Formally, the name 'CAMAC' denotes a set of standards for computer interface equipment. Informally it is applied to 'CAMAC equipment' which conforms to these standards and to 'CAMAC systems' assembled from this equipment. The CAMAC standards were originally developed to meet the needs of large-scale data acquisition systems for nuclear physics research. There have been other large-scale applications in computer control of processes and plant in industry and laboratories.

The following description of CAMAC is concerned mainly with the important but less spectacular field of application to small or medium computer systems for automated control and data-acquisition in a wide variety of laboratories. On-line systems involve connections between the computer and various control and measuring devices. These may use analogue signals (voltage, frequency, time, pulse-rate, and so on) or digital signals. The connection to the computer must use the particular input-output interface standard adopted by the computer manufacturer. CAMAC offers a powerful and flexible means of matching the differing standards of the computer and the external devices.

The basic principle of CAMAC (Fig. 1) is an assembly of plug-in modular units, one of which is connected to the computer and the others to various external devices and signals. The units are linked by a multi-wire highway (the CAMAC Dataway) which is an integral part of the chassis housing the plug-ins. Each plug-in occupies a multiple of 1/25th of the width of the chassis (Fig. 2), and generally has front-panel connections to external devices and signals, and rear connections to the Dataway highway. The Dataway carries signals for digital data and for control of data transfers, and also distributes power. The specification defines the mechanical dimensions of the plug-ins and their interface to the highway in just

sufficient detail to ensure that any plug-in will fit into the chassis and communicate by the highway. It leaves complete freedom to design plug-ins with a wide variety of facilities and external interfaces, and to use appropriate materials, components and styling. A typical highway operation takes 1 μ s to address a plug-in module and transfer to or from the computer a data word containing up to 24 bits. This is faster than the sustained data rate of most computers, but slow enough to be technically conservative.

The symmetrical name 'CAMAC' was chosen to symbolise this assembly of plug-ins facing in one direction to the computer and the other to the external devices. The name is now usually assumed to stand for 'computer automated measurement and control', which is a good indication of the main areas of application.

The CAMAC standards were developed by an international group representing users' interests in developing and applying laboratory instrumentation systems. The main standards¹⁻³ and other subsidiary standards were published by the Commission of the European

Communities, and were also published in identical form in the US. They are now being re-issued by the International Electrotechnical Commission and by various national standards organisations.

CAMAC is a standard that encourages manufacturers to produce compatible products which can then be assembled into systems by or for the users. It has led to the commercial production of over 1,000 plug-ins, together with supporting products such as the chassis, by 29 firms in many countries, often with the assistance and involvement of major users.

The main strength of CAMAC lies in the many hundreds of different types of plug-ins that face outwards to the external devices and signals. The functional characteristics and external interfaces of these plug-ins are determined by the users' needs and by market forces, rather than by the CAMAC specification. They range from quite simple units in one module of width through to highly complex units that may occupy two or more modules of width and may include a micro-processor. Some indication of the rich variety of these units can be seen from the following examples:

- General purpose units to accept or deliver digital signals, typically following CAMAC recommended practice for front-panel interfaces based on TTL circuit technology, and so suitable for use with the majority of modern items of equipment.

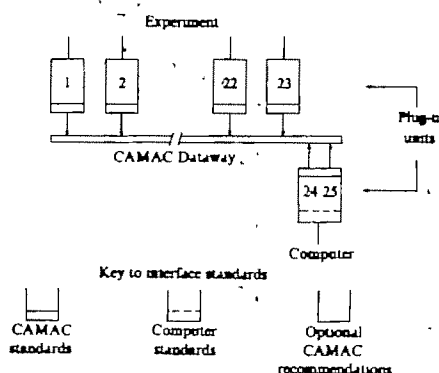
- Units to accept or deliver specialised signals, such as peak-sensing analogue-to-digital converters for the nuclear field.

- Units that are instruments in their own right, such as digital voltmeters in CAMAC units.

- Units that communicate with external instruments, for example through parallel digital interfaces or the General Purpose Instrument Bus.

- Units that are connected by communication links to peripherals such as teletypes or VDUs, or to a host computer.

Fig. 1 Basic small CAMAC system



Although each of these plug-ins is associated with a particular class of external device or signal it can be used with any computer. The basic costs of the mechanical parts and Dataway interface can be shared by several repeats of the same function within the same plug-in. Thus there are single-width CAMAC plug-ins containing, for example, 12 counters, 16 analogue-to-digital converters, or 8 digital-to-analogue converters.

A wide choice of plug-ins is also available for connecting CAMAC to the computer. Here four different strategies have been adopted, to cover varying requirements for the system configuration: (1) in small stand-alone systems the CAMAC plug-in can include a micro-processor; (2) in small systems with a separate computer, the CAMAC plug-in has an external interface to match the I/O standards of a particular computer (Fig. 1). This arrangement is widely used, and plug-ins are available to match most popular minicomputers; (3) in larger compact systems not exposed to electrical interference the plug-in can be connected to the computer by a CAMAC parallel highway (Fig. 3). This can serve seven CAMAC assemblies, and there are units to couple it to many popular computers; (4) in larger dispersed systems that are exposed to interference the plug-in can be connected to the computer by a CAMAC serial highway (Fig. 3), which can serve 62 CAMAC assemblies.

Although some of these units couple CAMAC to one specific type of computer they can be used with any of the plug-ins that interface to external devices and signals. In typical configurations of CAMAC systems (Figs 1 and 3) all the internal interfaces conform to CAMAC standards, and many of the external interfaces follow CAMAC recommendations.

With falling costs of computers and memory, the computer program specific to a particular application has become a major part of the system cost, especially in 'one off' systems. In any on-line system the software must control input-output through the interface. In the case of CAMAC all I/O operations are controlled by unified commands which specify the plug-in module, a subsection of the plug-in (such as one of the 12 counters, 16 ADCs or 8 DACs

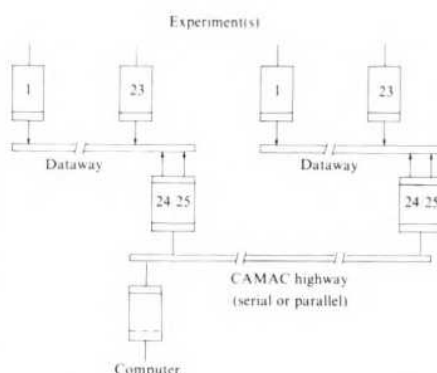


Fig. 3 Larger CAMAC system. See Fig. 1 for key to interface standards.

mentioned above) and the type of operation (such as reading to the computer or writing from the computer). As these commands are uniform for all plug-ins from all manufacturers this helps to simplify and rationalise the software. Several different recommendations for CAMAC I/O software have been published and implemented, to take advantage of the varying needs and skills of the users. First there are recommendations⁴ for macro-instructions for systems programmed in assembly language, an approach often adopted by larger users with appropriate staff. Then there are recommendations for sub-routines⁵, typically to take advantage of the FORTRAN programming experience of scientists. Third, there is an extended form of BASIC⁶ which requires little previous experience of programming or CAMAC. Another derivative of BASIC is well established in the UK for testing CAMAC equipment and for small-scale systems. These four well-documented approaches to CAMAC software provide overlapping coverage of a wide range of situations.

As a multi-national standard that is not tied to any particular firm or type of computer CAMAC has the advantage of a multiplicity of

suppliers, so that the availability of equipment for replacements and extensions is not subject to the commercial policy or survival of any one firm. The wide range of commercially available equipment makes it possible to assemble systems with the minimum of hardware development. At one extreme, the most frequently needed equipment is usually available from several competing suppliers. At the other extreme, equipment to meet some specialised requirement can often be found by searching the published worldwide guide of CAMAC products⁷. Software products are readily available, but generally on an informal or semi-commercial basis from large user organisations.

CAMAC is not a standard that impedes progress—it deliberately allows freedom to exploit new technology such as improved integrated circuits and microprocessors, and to provide the facilities and configurations needed by the user. CAMAC is seen to have considerable advantages in flexibility, versatility, generality and availability when compared with other established methods of interfacing, such as computer manufacturers' interface equipment, specially designed equipment, and the General Purpose Instrument Bus used on many instruments.

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AERE Harwell,
Oxon, UK

1. ESONE, EUR 4100e (CEC, Luxembourg, 1972).
2. ESONE, EUR 4600e (CEC, Luxembourg, 1972).
3. ESONE, EUR 6100e (CEC, Luxembourg, 1977).
4. ESONE/IML/01 (CEC, Geel, Belgium, 1974).
5. DOE/EV-0016 (Washington DC, 1978).
6. ESONE/RTB/02 (CEC, Geel, Belgium, 1977).
7. European CAMAC Ass. (CEC, Geel, Belgium, 1978).

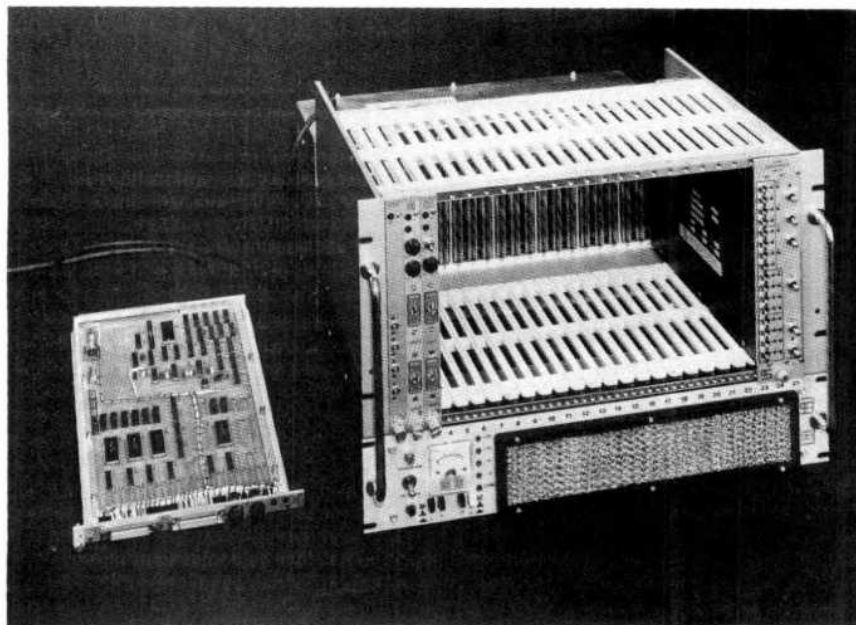
A worked example of system design

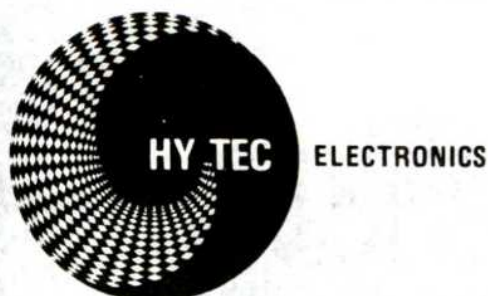
Using the CAMAC standards as a basis for laboratory system design, provides practical benefits for the scientist who requires real-time computing power in his research. This can be illustrated by working through the design and implementation of a specific example.

The particular requirement concerned a data acquisition system for a gamma camera, for a large hospital in Belgium. The primary function of the system was to interface to the camera which produces two analogue pulses that have to be amplitude analysed, and the resulting data transferred to a host processor 150 m away. The processor's task was to generate full colour graphic displays of the collected data, and the design of the acquisition system permitted dynamic studies of the camera output to be made with far better time resolution than previously possible. The overall system now gives the doctors not only an improved diagnostic capability on existing camera measurements, but also provides new diagnostic performance on dynamic studies, at the same time generating very significant savings in operating costs over the present photographic methods of recording camera data.

The acquisition system plays only a small, albeit crucial, role in the overall system. It must therefore not only meet the specific

Fig. 2 CAMAC chassis and plug-in units.





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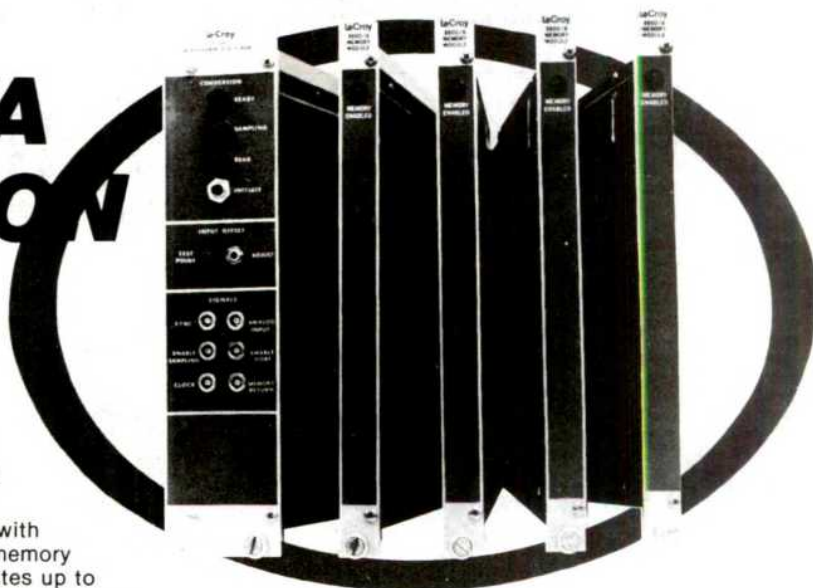
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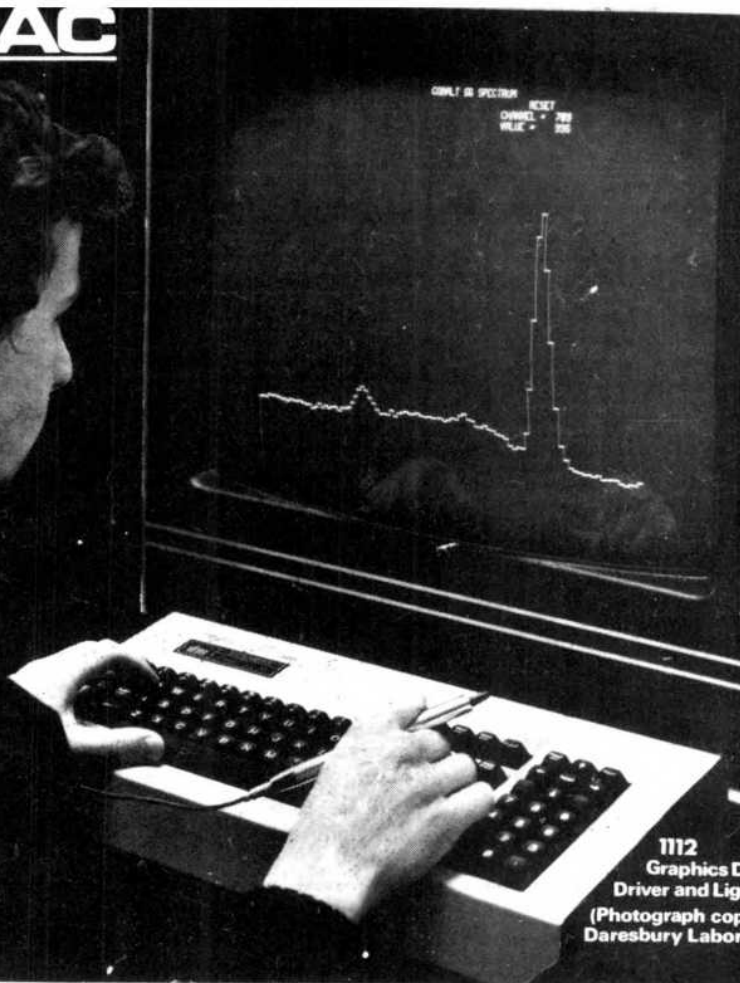
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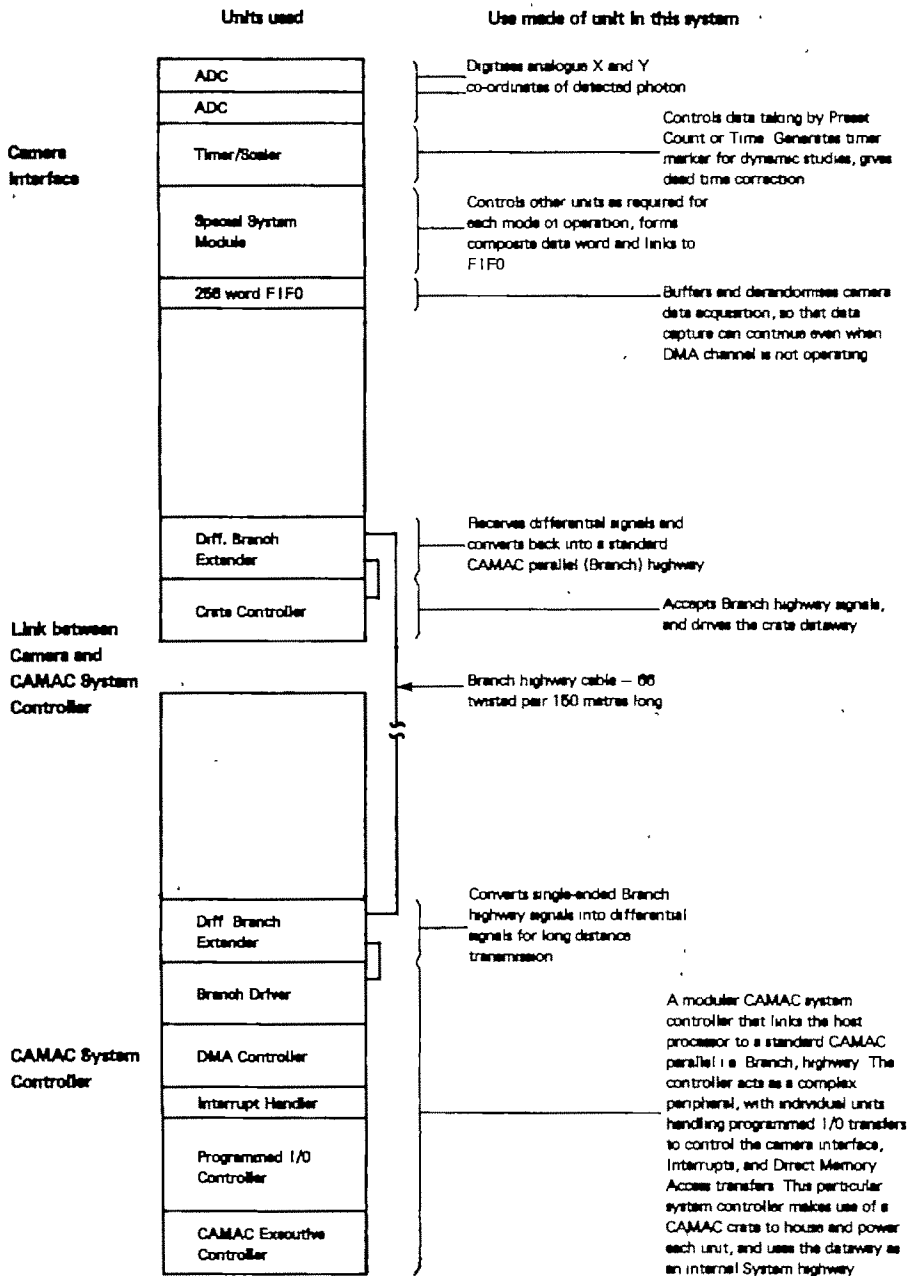


Fig. 1 Block diagram of gamma camera data acquisition system.

performance objectives, but must also be easy to use, maintain and develop as new requirements emerge, whilst minimising costs and implementation time scales at all times.

The starting point in the design of a system to meet this requirement was to base it on CAMAC equipment. In this specific case the decision proved to be readily justifiable, as the system was implemented almost entirely from standard CAMAC products. This was not possible with any other interface scheme. One other approach often considered is the design and construction of a special purpose system. Such an approach raises many fundamental questions of system structure, such as the size of the basic module, the power supply requirements, the way in which each unit relates to the others and so on, none of which have direct relevance to implementing the actual system, but all of which can have a vital impact on the ease with which the system is commissioned, used and maintained, and its flexibility in

copling with new requirements. CAMAC provides a rigorously defined system framework within which the designer is free to concentrate on solving the specific problems posed by the requirements, whilst at the same time building in flexibility into the basic design process. The special design approach was in fact ruled out by the hospital, as they neither had, nor wanted, the level of electronic expertise required to implement a custom-built system. The block diagram of the system is shown in Fig. 1, and divides naturally into three parts; the camera interface, the computer interface, and the link between the two. The requirements for connecting to the camera were very particular to both the specific model of camera used, and the types of measurements required from the system. However, most of this unique requirement was met by a combination of currently available CAMAC units. The special unit acts mainly as a mechanical interface for the cabling between the other units. It contains

only about 20 integrated circuits that control the interconnections in the manner required to achieve the overall interface function. Note that units made by four different companies are used. The system designer is not restricted by the competence of any one supplier. Indeed it is not unusual to find one CAMAC supplier with special competence in analogue unit design, whilst another specialises in the rather different, but equally complex discipline of computer interfaces to CAMAC. Total control of the camera interface is through the CAMAC Dataway, and so it can in principle be controlled by any CAMAC Dataway controller, from a microprocessor resident in the same assembly, to any of the system configurations the CAMAC standards have defined.

The computer interface requirements for this system were more complex than for many laboratory systems. In addition to the normal programmed I/O to control the data acquisition, data transfers have to be at high speed, directly into the host computer's store, and in either List mode (one word at a time transferred into successive memory locations) or in Spectrum mode (the data word is used to address a memory location, whose contents are automatically incremented). Even so the system could be constructed entirely from available CAMAC units, and needed no special design effort. The benefit can be more readily appreciated by the realisation that the unit design effort embodied in just this part of the system represents several man-years of work. Finally the standard CAMAC parallel highway, extended by readily available extender units, was used to provide the required high-speed link over the 150 m between the computer and the camera. For the majority of the units selected in the whole design, the system designer had a choice of several different suppliers. For example, the Crate Controller could have been obtained from at least 10 other companies. Neither would the system design have been significantly affected had the host processor been one of a very substantial range of other types, as most of the system is independent of the computer type, and suitable computer interfaces to the CAMAC parallel highway are available for many different models of computer. That it was possible to meet this somewhat unusual system requirement using available CAMAC units, is a good indication of the scope and range of CAMAC products that are now available to the system designer.

The 'acid test' of any system structure is the ease, or otherwise, with which individual elements designed to be compatible with the system can in practice be combined into a working system. Experience over the past eight years has shown the CAMAC standards to be soundly based, with the integration of individual units into a working system remarkably free of those 'unexpected' interactions that can often affect less completely specified structures. In the case of the camera application this was demonstrated by the ease with which the various manufacturers' units were incorporated. System commissioning was also greatly assisted by the availability of system test and diagnostic equipment, and particularly by the availability of system test software for most of the individual units. The only special test software required was a set of short programs to establish that the system performed the overall functions correctly. All of the diagnostic and test software is written in a high-level BASIC-like language, enabling the user to take advantage of these packages for system maintenance.

The lack of these support facilities can extend commissioning and fault-finding times very significantly.

As a direct consequence of employing commercially available products, the documentation on the individual units is usually to a high standard. The system designer is left with the relatively straightforward task of describing the interaction between these items, and the overall system functions. Good documentation, however, is essential if a system is to remain serviceable in the medium term. It is not uncommon to find equipment that has had to be scrapped once the originator was no longer available to maintain it, because the documentation was never produced. Of course, problems in system design do not end with the commissioning of the system, even assuming that it does fully meet the user's requirements. The system has to be kept in service, and so ease of maintenance can be an important system design consideration. Certainly this was true of the camera system, and the CAMAC approach addresses this problem in several ways. For example, the structure is modular along functional lines. This simplifies the isolation of a fault to a particular unit, and allows first-line maintenance by unit replacement. The system-test software and hardware mentioned above also simplify this process. Faulty units can usually be returned to the supplier for rectification, thus taking advantage of their sophisticated automatic test equipment. As CAMAC equipment is often sold as individual products to system builders, items are often available either 'off the shelf' or on short delivery, and this can act as a valuable back-up to the normal system spares.

Initial system design often makes little or no allowance for the further development of the system. With the CAMAC approach the flexibility to cope with the needs for expansion and up-grading is built into the system structure. Since the initial camera system was commissioned, several additional requirements have been considered, that illustrate this point. The first is to connect the system to three other cameras over the next year or so. As the computer interface will support up to 49 CAMAC chassis, there is in practical terms no physical limit to the number of cameras that could be connected. The second is possibly more interesting, in that it demonstrates how the CAMAC standards have managed to evolve and anticipate new system requirements. As more cameras are added, the host processor will not be able to cope with the instantaneous data rates being generated, and the intention is to add local processing to each camera interface to preprocess the data. The most recent CAMAC standard covers exactly this requirement, and microcomputers are already available that will go into the camera interface assembly and provide the required function.

One final benefit that came from the use of CAMAC in this system was the genuine assistance the hospital staff were able to get from the CAMAC user community in Belgium, in terms of providing basic training and familiarity with CAMAC.

I thank Professor C. Beckers and M. Steels of the Department of Nuclear Medicine, of the Hospital UCL-St Luc, for presenting this interesting problem for us to solve and the directors of GEC-Elliott Process Automation Limited for permission to publish this article.

D. M. DRURY

GEC-Elliott Process Automation Ltd,
Leicester, UK

CAMAC applications in biological systems

The application of CAMAC to biology has been given an impetus in recent years by the increasing use of large scale experimental facilities producing neutron or photon probes. Data rates may be high and to make the best use of these facilities it becomes of paramount importance that experiments should be highly automated.

From the point of view of such laboratories CAMAC provides a very good solution to several requirements. The systems must be flexible as they may be required to adjust to new experiments every few days so that the hardware must clearly be modular. It must also be largely available commercially because, though there will always be special units that must be designed 'in-house', the total quantity of units required will usually exceed the local design and production capacity of the laboratory. Maintenance problems are simplified as is software implementation as the use of a standard forms a well understood bridge between the programmer or maintenance engineer and the application.

These are general points that apply to systems outside biology as well as within it. Furthermore, though they may seem to be less pressing, these considerations arise also for the individual experimenter implementing a system at his home laboratory.

Several laboratories are using CAMAC to support experiments in biology. At the high flux neutron reactor of the Institut Laue Langevin (ILL) at Grenoble CAMAC output registers are in use for positioning stepper and servo motors on diffractometers and monochromators. Small Angle Scattering (SAS) cameras at ILL use incrementing CAMAC memories to interface 2-D detectors with up to 128×128 cells.

The European Molecular Biology Laboratory (EMBL) is standardising the use of CAMAC and it is in use at the main centre at Heidelberg and their outstations at the electron synchrotron DESY at Hamburg and the ILL. Among its present and planned uses are for experiments on fly flight dynamics, EXAFS (extended X-ray absorption fine structure) experiments, centrifuges, stepper motors and polar coordinate X-ray detectors. Three film scanners are interfaced by CAMAC to two Nord-10 computers and a PDP-11/45 and two film writers to Nord-10s. At the DESY outstation the CAMAC serial highway is used to connect two experimental areas separated by some 300 m.

On the electron synchrotron NINA (now closed) at Daresbury, monochromators were also positioned by CAMAC stepper motor drivers and monitored by shaft encoders. Incremental encoders were interfaced by CAMAC up-down binary counters and absolute encoders by input registers. CAMAC 24-bit pre-set scalars were used to record data from ion chambers on EXAFS experiments. An electron storage ring, known as the SRS, is being constructed at Daresbury as a dedicated source of synchrotron radiation, and it is due to become operational in Spring 1980. The data acquisition system will use CAMAC (as does the control system). Among the instruments planned for the SRS are multiple solid-state

detectors. They are being made for energy-dispersive SAS experiments and high rates ($100\text{K counts s}^{-1}$) are desired from each detector. It is intended that incrementing CAMAC memory be used for the data accumulation.

The MRC (Medical Research Council) at Cambridge is developing an experiment using CAMAC to study the dynamic response of muscle to a stimulus. The magnitude of intensity peaks on a linear position sensitive detector will be studied over the 200 ms or so duration of a twitch with the aid of 100 buffered scalars. The scalar buffer registers will be read out every millisecond into a 32K word CAMAC memory while counting proceeds. To achieve the required high rate of transfers on the CAMAC dataway a simple auxiliary crate controller will be used to transfer data from the scalars to the memory.

We hope that these few examples have shown that the same units, stepper motor drivers, memories and scalars, for instance, are relevant to several different experiments. Note too that in CAMAC these units are computer independent it is apparent that an experimenter wishing to set up a new instrument or experiment will find that most if not all of the interfacing problems are already solved. As more systems are implemented in CAMAC the number of shareable and transportable solutions to problems in experimental biology will continue to grow. Daresbury intends to encourage this by loaning CAMAC systems to prospective users of the SRS. They may then develop instruments and experiments in advance of bringing them to the SRS and establish systems at their home laboratories which are compatible with those at the SRS.

To complement the ease with which CAMAC systems may be configured it is highly desirable to provide software that may be as easily written. CAMAC assists here too as the use of standard hardware promotes standard hardware-handling facilities in the software. Several different approaches to this have been adopted. At Daresbury a version of BASIC has been developed with extensions to drive CAMAC efficiently. This will make it possible for the biologist himself to write the programs to control his experiment. The extended BASIC compiler, called CATY, is also in use at EMBL, DESY enabling the two laboratories to share programs. This has happened in the case of EXAFS experiments even though different computers are in use.

As CAMAC finds application in the major facilities, techniques and equipment will evolve that match the many new applications in the area of biological science. This will lead to the availability of equipment that will be relevant also to the small biology laboratory and the scientist will be able to take advantage of the application of computing and modular electronics. This should enable scientists to use data acquisition and experiment control systems employing mini and micro computers in the study of biology without having to devote a significant proportion of their time to problems of electronics.

We thank Drs P. N. Clout of EMBL, R. Ghosh of ILL and A. R. Faruqi of the MRC, Cambridge for providing information used here.

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General

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ROSEN, Robert. *Fundamentals of Measurement and Representation of Natural Systems*. (The North-Holland Series in General Systems Research.) Pp. xvii + 221. ISBN-0-444-00261-8. (New York: Elsevier North-Holland, Inc., 1978. Distributors outside the U.S.A. and Canada: Thomson Books, a Division of Elsevier/North-Holland Scientific Publishers, Ltd., P.O. Box 85, Limerick, Ireland.) £22.95.

SLOMAN, A. *The Computer Revolution in Philosophy: Philosophy Science and Models of Mind*. (Harvester Studies in Cognitive Science.) Pp. xvi + 304. ISBN-0-85527-389-5. (The Harvester Press: Sussex, 1978.) £12.50.

STUDENTS IN NEED. (Essays in Memory of Nicolas Malloson.) Pp. 230. ISBN-0-900868-60-0. (Guildford, Surrey: Society for Research into Higher Education Ltd., at the University of Surrey, 1978.) £5.50.

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ZAJIC, J. E., and HIMMELMAN, W. A. *Highly Hazardous Materials Spills and Emergency Planning*. (Hazardous and Toxic Substances: a Series of Reference Books and Textbooks, Vol. 1.) Pp. x + 225. ISBN-0-8247-6622-9. (New York and Basel: Marcel Dekker, Inc., 1978.) Sw. francs 65.

announcements

AWARDS

The Columbia University's 1978 Louisa Gross Horwitz Prize has been awarded to **David H. Hubel** and **Torsten N. Wiesel** (Harvard University), and **Vernon B. Mountcastle** (The Johns Hopkins University), who will share the award (\$25,000) equally for their contributions to our understanding of the brain.

The Institute of Food Science and Technology has awarded the Mounfield Prizes to **S. R. Tomlinson** (National College of Food Technology) and **Dut Fan Chung** (Polytechnic of the South Bank).

A limited number of Research Fund grants, each of £200, will be awarded by the Chemical Society for the year 1979. Application forms, together with the regulations governing the Fund, may be obtained from Dr Malcolm D. Robinson, The Chemical Society, Burlington House, London W1, UK. The closing date for applications is 1 November 1978.

The Fifth Marconi International Fellowship Award will be given to a person who has made outstanding advances in satellite and space technologies relevant to improving world communications, according to Walter Orr Roberts, Fellowship Secretary. Nominations should be submitted before 15 November, 1978, to the Marconi International Fellowship, Aspen Institute, 1919 14th Street, No. 811, Boulder, Colorado 80302.

MEETINGS

4 December, **Prospects for Power from Ocean Waves**, Edinburgh (Executive Secretary, The Royal Society of Edinburgh, 22 & 24 George Street, Edinburgh, UK).

5 December, **Vibrational Spectroscopy in Inorganic Chemistry**, London (Stanley Langer, The Chemical Society, Burlington House, Piccadilly, London W1, UK).

6-7 December, **The Evolution of Adaptation by Natural Selection**, London (Executive Secretary, The Royal Society, 6 Carlton Terrace, London SW1, UK).

9 December, **Tay Estuary Symposium**, Dundee (Dr J. McManus, Department of Geology, The University, Dundee, UK).

12-13 December, **The Middle Atmosphere as observed from Balloons, Rockets and Satellites**, London (Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1, UK).

9-11 April 1978, **Metal Ion Transport and Storage**, Sheffield (Professor P. M. Harrison, Department of Biochemistry, University of Sheffield, Sheffield, Yorks, UK).

17-21 April, **Special FEBS Meeting on Enzymes**, Dubrovnik (Secretariat: Laboratory of Biochemistry, Technological Faculty, Pierottijeva, 6/V1, YU-41000 Zagreb, Yugoslavia).

20-21 April, **Recent Advances in the Study of British Flora**, Manchester (Miss L. Shore, Department of Botany, The University, Manchester, UK).

28 April-2 May, **81st Meeting of the American Ceramic Society**, Cincinnati (R. L. Berger, 3211 Civil Engineering Building, University of Illinois, Urbana, Illinois 61801).

7-9 May, **The Role of Peptides in Neuronal Function**, Bethesda (Dr Barker, Building 36, Room 2C02, NIH, 9000 Rockville Pike, Bethesda, Maryland 20014).

20-21 June, **New Horizons in Industrial Microbiology**, London (Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1, UK).

27-31 August, **The Role of Water in Urban Ecology**, Amsterdam (K. C. Plaxton, PO Box 330, Amsterdam, The Netherlands).

2-7 September, **7th International Meeting of the International Society for Neurochemistry**, Jerusalem (Marco Trabucchi, Ente Universitario Lombardia Orientale, Istituto di Farmacologia e Terapia, Via Valsabbina, 19, 25100 Brescia, Italy).

3-5 September, **Erosion by Liquid and Solid Impact**, Cambridge (5th International Conference on Erosion by Liquid and Solid Impact, Cavendish Laboratory, Madingley Road, Cambridge, UK).

3-6 September, **EMAG 79**, Sussex (The Meetings Officer, The Institute of Physics, 47 Belgrave Square, London SW1, UK).

3-7 September, **Insect Neurobiology and Pesticide Action**, York (The Society of Chemical Industry, 14 Belgrave Square, London SW1, UK).

5-9 September, **15th International Congress of Therapeutics**, Brussels (Secretariat of the Congress, Department of Cardiology, Hopital Universitaire Saint-Pierre, rue Haute 322, 1000 Brussels, Belgium).

10-13 September, **1st International Symposium on Neuroactive drugs in Endocrinology**, Milan (Eugenio E. Muller, Department of Pharmacology, University of Milan, 32 Via Vanvitelli, 20129 Milan, Italy).

12-13 September, **How will tomorrow's microprocessor based process instrumentation communicate?** London (Miss Audrey Mills, Sira Institute Ltd, South Hill, Chislehurst, Kent, UK).

16-21 September, **4th Conference on Photographic Gelatin**, Oxford (H. A. Adam, Processing Technology Laboratory, Kodak Ltd, Headstone Drive, Harrow, Middlesex, UK).

17-20 September, **2nd International Reindeer/Caribou Symposium**, Røros (E. Reimers, Institute of Zoophysiology, University of Oslo, Box 1051 Blindern, Oslo 3, Norway).

17-21 September, **7th International Congress of Pathology**, Valencia (Professor A. Lombart-Bosch, Department of Pathology, Medica School, Valencia Apartado 2045, Spain).

19-21 September, **4th National Quantum Electronics Conference**, Edinburgh (Dr B. S. Wherrett, Physics Department, Heriot-Watt University, Edinburgh, UK).

25-27 September, **Organisation of Macromolecules in the Condensed Phase**, Cambridge (Mrs Y. A. Fish, Faraday Division, The Chemical Society, Burlington House, London W1, UK).

The previous list of meetings was in *Nature* of 19 October, page 679.

Reports & Publications

UK & Ireland—March

University of Bristol: Department of Agriculture and Horticulture. Long Ashton Research Station, The National Fruit and Cider Institute—Report 1977. Pp.xvi+243. (Long Ashton, Bristol: Long Ashton Research Station, University of Bristol, 1978.) £2.50. [1910]

University College London. Calendar, 1978-1979. Pp.238. (London: University College London, 1978.) [1910]

Social Science Research Council. Research Supported by the Social Science Research Council 1978. Pp.vi+440. (London: Social Science Research Council, 1 Temple Avenue, EC4, 1978.) [1010]

The Nuffield Foundation. Report for the year 1977. Pp.113. (London: The Nuffield Foundation, Nuffield Lodge, Regent's Park, NW1, 1978.) [1010]

Open Earth, No. 1, October 1978. Edited by Dr. Peter J. Smith. Published quarterly. Pp.1-52. (Hanslope, Milton Keynes: *Open Earth*, 32 St. James Close, 1978. Subscription rates available from above address.) [1010]

Liquid Fuel from Coal. (A National Coal Board Report prepared by Planning, Assessment and Development Branch of the Coal Research Establishment, with Operational Research Executive.) Pp.70. (London: National Coal Board, Hobart House, Grosvenor Place, SW1, 1978.) £7.50. [1010]

Employment and Training in the Motor Vehicle Industry. By Peter Brayshaw and Jane Cook. (EITB Reference Paper.) Pp.116. (Watford: Engineering Industry Training Board, 54 Clarendon Road, 1978.) [1010]

Tropical Products Institute Seminar on Post-Harvest Grain Losses. Edited by Mrs. S. P. D. Stokes. (Tropical Stores Products Information, No. 36.) Pp.xvii+72. (Slough: Tropical Stores Products Centre, Tropical Products Institute, London Road, 1978.) £1.60. [1610]

British Standards Institution. Annual Report, 1977 to 1978. Pp.16. (London: British Standards Institution, 2 Park Street, W1, 1978.) [1610]

Food, Health and Farming: Reports of Panels on the Implications for UK Agriculture. Edited by C. J. Robbins. (CAS Paper No. 7.) Pp.119. (Reading: Centre for Agricultural Strategy, University of Reading, 2 Earley Gate, 1978.) [1810]

Scottish Marine Biological Association. Annual Report for the year ending 31st March 1978. Pp.89. (Oban, Argyll: Scottish Marine Biological Association, Dunstaffnage Marine Research Laboratory, 1978.) £1. [1810]

University of Oxford. Oration by the Vice-Chancellor and Annual Report, 1977-1978. Pp.89-111. (Oxford: The University, 1978.) 20p. [1810]

British Nutrition Foundation. Annual Report, 1977-1978. Pp.24. (London: British Nutrition Foundation, 15 Belgrave Square, SW1, 1978.) [1910]

Other countries—March

Proceedings of the Nutrition Society of Australia. Vol. 3: Third Annual Conference, Adelaide, South Australia, August, 1978. Pp.111. (Blacktown, N.S.W.: Secretary, Nutrition Society of Australia, CSIRO, Division of Animal Production, PO Box 239, 1978.) A\$5. [510]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2046: Calibration of a Mathematical Model of the Antelope Valley Ground-Water Basin, California. By Timothy J. Durbin. Pp.v+51+13 plates. (Washington, DC: US Government Printing Office, 1978.) [510]

Académie Royale de Belgique. Mémoires de la Classe des Sciences. Collection in-8—2^e Série. T. XLIII, Fasc. 1: Nouvelles Recherches sur les Manifestations Vibratoires des Interactions entre Molécules ou Groupes d'Atomes dans les Solides. Par M. J. Pottier. Pp.74. T. XLIII, Fasc. 2: Contribution à l'Étude du Transport Électrique dans les Alliages de Métaux de Transition et de Métaux Nobles à Forte Teneur. Par Michel Brauwers. Pp.112. (Bruxelles: Académie Royale de Belgique, 1978.) [910]

Bulletin of the American Museum of Natural History. Vol. 161, Article 1: Type Specimens of Birds in the American Museum of Natural History. Part 2. By James V. Greenway, Jr. Pp.1-306. (New York: American Museum of Natural History, 1978.) \$20.45. [910]

Fisheries and Environment Canada: Fisheries and Marine Service. Technical Report No. 785: Physical Characteristics of Atlantic Salmon Spawning Gravel in some New Brunswick Streams. By R. H. Peterson. Pp.iv+28. (St. Andrews, N.B.: Biological Station, 1978.) [1210]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 16079: Ecotoxicology of Chlorpyrifos. By W. K. Marshall and J. R. Roberts. Pp.314. (Ottawa: National Research Council Canada, 1978.) \$2. [1210]

Geological Survey of Canada. Bulletin 266: Carboniferous Biostratigraphy and Correlation, Northeastern British Columbia and Southeastern District of Mackenzie. By E. W. Bamber and B. L. Mamet. Pp.65 (6 plates). Bulletin 267: Contributions to Canadian Paleontology (five papers). By B. S. Norford, et al. Pp.75 (14 plates). \$5.10. Bulletin 276: Stratigraphy and Correlation of Lower Paleozoic Formations, Subsurface of Cornwallis, Devon, Somerset, and Russell Islands, Canadian Arctic Archipelago. By Ulrich Mayr. Pp.55. \$6. Bulletin 302: Devonian Stratigraphy, West-Central Ellesmere Island, Arctic Archipelago. By H. P. Trettin. Pp.119. \$6. (Ottawa: Geological Survey of Canada, 1978.) [1210]

National Research Council Canada. Associate Committee on Scientific Criteria for Environmental Quality. NRCC No. 15391: Effects of Arsenic in the Canadian Environment. Pp.349. (Ottawa: National Research Council Canada, 1978.) \$2. [1310]

New Zealand: Department of Health. Environmental Radioactivity Annual Report, 1977. Pp.12. (Christchurch: National Radiation Laboratory, PO Box 25-099, 1978.) [1310]

Studia Forestalia Suecica, Nr. 145: Ultrastructural Studies of Resistance Mechanisms in *Pinus sylvestris* L. Against *Melanospora pinitroqua* (Braun) Rostr. (Pine Twisting Rust). By Alena Jonsson, Monica Holmvald and Björn Wallen. Pp.28. (Uppsala, Sweden: The Swedish University of Agricultural Sciences, College of Forestry, 1978.) [1710]

Australia: Commonwealth Scientific and Industrial Research Organization. Division of Mechanical Engineering—Report on Research 1975-77. Pp.80. (Melbourne: CSIRO, 1978.) [1710]

United States Department of the Interior: Geological Survey. Water-Supply Paper 2047: Geology and Ground Water in Door County, Wisconsin, with Emphasis on Contamination Potential in the Silurian Dolomite. By M. G. Sherrill. Pp.v+38+5 plates. Professional Paper 950: "Nature to be Commanded..." Earth-Science Maps Applied to Land and Water Management. By G. D. Robinson and Andrew M. Spieker. Pp.95. (Washington, DC: US Government Printing Office, 1978.) [1710]

United States Department of the Interior: Geological Survey. Bulletin 1443: Feasibility and Cost of Using a Computer to Prepare Landslide Susceptibility Maps of the San Francisco Bay Region, California. By Evelyn B. Newman, Arthur R. Paradis and Earl E. Brabb. Pp.iii+27+1 plate. Professional Paper 813-M: Summary Appraisals of the Nation's Ground-Water Resources—Hawaii Region. By K. J. Takasaki. Pp.iv+29. (Washington, DC: US Government Printing Office, 1978.) [1810]

Principles and Methods for Evaluating the Toxicity of Chemicals, Part 1. (Environmental Health Criteria, 6). Pp.272. Published under the joint sponsorship of the United Nations Environment Programme and the World Health Organization. (Geneva: WHO; London: HMSO, 1978.) Sw.fr.28; \$15.40. [1910]

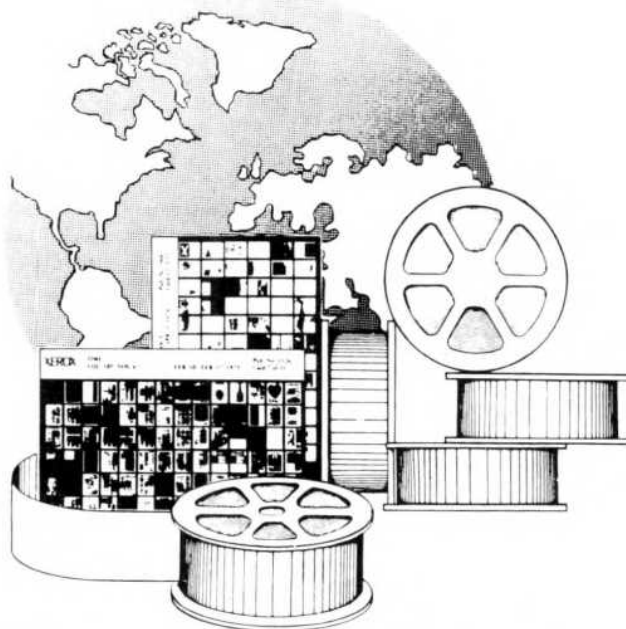
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UNIVERSITY OF RHODESIA DEPARTMENT OF CROP SCIENCE

Applications are invited for the post of
**SENIOR LECTURER/
LECTURER IN PLANT
BREEDING AND GENETICS**

Preference will be given to applicants with relevant postgraduate qualifications and experience. The appointee will be required to undertake research in the breeding of more productive crops in such crop species as groundnuts, sweet potatoes and other food crops which are cultivated mainly by peasant farmers and farmers in a developing agricultural sector. The appointee will be required to assist with teaching in Genetics and Plant Breeding courses in the B.Sc. Agriculture with honours degree and assist in the supervision of research students reading for higher degrees. This post will be funded for an initial period of three years. It is hoped that the successful applicant will take up the appointment in March 1979.

Salary Scales (approx. stg. equiv.): Senior Lecturer, £7,544 by 292 to £8,423 by 310 to £9,973; Lecturer Grade I, £6,843 by 257 to £7,871; Lecturer Grade II, £4,224 by 222 to £5,334 by 257 to £6,619.

Applications: (6 copies) giving full personal particulars (including full names, place and date of birth, etc.), qualifications, experience and publications and names and addresses of three referees should be submitted by 15 January 1979, to the Registrar, University of Rhodesia, P.O. Box P 167, Mount Pleasant, Salisbury, Rhodesia, from whom further particulars may be obtained.

Overseas applicants should send a full copy of their applications to the Association of Commonwealth Universities (Appis), 36 Gordon Square, London WC1H 0PF, from whom further particulars may also be obtained.

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 233 4143) or our nearest British Consular Office. 8182(A)

THE MEDICAL COLLEGE OF ST BARTHOLOMEW'S HOSPITAL

DEPARTMENT OF EXPERIMENTAL PATHOLOGY

Research Assistant required to work on basic research into inflammation and rheumatology for a two year period, with a possibility of working towards a higher degree. Salary Scale £3,384 to £4,882 plus £450 London allowance. Further particulars may be obtained from Professor D. A. Illingworth. Applications should be sent to The Secretary, The Medical College of St Bartholomew's Hospital, East Smithfield, London EC1A 7BE. Closing reference 806 8091(A)

ROYAL FREE HOSPITAL SCHOOL OF MEDICINE (University of London)

Applications are invited for the post of
LECTURER

in the PHYSIOLOGY DEPARTMENT from January 1, 1979 or as soon after as possible. Salary on scale £3,909 to £7,754 plus £450 London Allowance. Further particulars from the Secretary of the School, 8 Hunter Street, London WC1N 1BP, to whom applications (six copies, including names of two referees) should be sent not later than November 18, 1978. 8129(A)

THE UNIVERSITY OF MANCHESTER

DEPARTMENT OF CHEMISTRY

Applications are invited as soon as possible for an S.R.C. Postdoctoral Fellow to study the kinetics and mechanisms of the reactions of electronic defects ("colour centres") in simple ionic solids such as alkali halides, using the technique of pulse radiolysis. The project is a continuation of on-going research in this area being supervised by Drs G. W. A. Newton and V. J. Robinson. Research experience in the solid state or in pulse radiolysis would be an advantage. Initial salary not less than £3,883 p.a. Superannuable. The post is for one year in the first instance with the possibility of renewal for a further year. Applications, including a curriculum vitae and the names of two referees should be sent to Dr V. J. Robinson, Chemistry Department, The University, Manchester M13 9PL (from whom further details may be obtained). 8130(A)

UNIVERSITY OF ABERDEEN

DEPARTMENT OF FORESTRY RESEARCH ASSISTANT IN FOREST ECOLOGY

Applications are invited for the above post. The successful applicant will work on a project to devise options for managing parts of certain native pinewoods of Scotland such that they may be conserved as important natural ecosystems yet yield sustained amounts of timber. The sponsor is Nature Conservancy Council. Qualifications: degree in Forestry, Biology, Ecology, Botany or Zoology. Principal investigator: Professor J. D. Matthews.

Salary on scale 1B £3,384 to £4,882 per annum. Duration 21 months from November 1978.

Further particulars from The Secretary, The University, Aberdeen, with whom applications (2 copies) should be lodged by November 17, 1978. 8108(A)

ANGLO-AUSTRALIAN OBSERVATORY RESEARCH ASTRONOMERS

The Anglo-Australian Observatory comprises the 3.9 metre telescope near Coonabarabran, N.S.W. and a laboratory at Epping near Sydney, Australia. There is a scientific staff of 6 astronomers, 3 of whom are nominated by Australia and 3 by the U.K. The 3 Australian and 1 of the U.K. positions become vacant between 1 July, 1979 and 1 January, 1980.

Candidates should have strong backgrounds in some branch of Physics or Astrophysics, including theory. Appointments will be fixed terms of 1 to 5 years, with salaries in the range \$A14,829 to \$A29,356 per year (Subject to review in 1979) plus other allowances, fares etc.

Applications should include:

1. Age, citizenship, country of residence and marital status.
2. Qualifications and experience, including date of Ph.D.
3. Astronomical research projects for which telescope time will be sought (sufficient information is required to show that the proposals would be competitive in applications to the panel for the allocation of telescope time).
4. Any instrument development project in which the applicant is particularly interested.
5. When applicant would be available.
6. Length of appointment envisaged.
7. Names and addresses of three referees.

Applicants should arrange for the three references to be sent directly to the Secretary by the closing date of 5th January, 1979.

Applications should reach the Secretary, Anglo-Australian Telescope Board at either Department of Science, P.O. Box 449, Woden, A.C.T. 2606, Australia or Science Research Council, P.O. Box 18, Swindon SN2 1ET, United Kingdom. 8142(A)

SHEFFIELD SOUTHERN HEALTH DISTRICT (TEACHING)

DEPARTMENT OF BACTERIOLOGY

A vacancy exists in the Group Department of Bacteriology situated at the Royal Infirmary, The Jessop Hospital for Women and Hallamshire Hospital for—

A NON-MEDICAL BACTERIOLOGIST

Basic Grade or Probationer

Applicants should possess a 1st or 2nd class Honours Degree in Biological Sciences and should have spent part, preferably the major part, of their course in Microbiology.

Whitley Council Conditions of Service apply.

Salary Scale: £3,486 to £4,275 per annum for new entrants to the Health Service.

Visits welcomed, by appointment with Mr J. M. Hindmarch, Principal Non-Medical Bacteriologist, Royal Infirmary, Sheffield S6 3DA (Telephone 0742-20977, ext. 326).

Applications stating age, qualifications and experience naming 2 referees to the District Personnel Officer, District Personnel Office, Floor 'B', Hallamshire Hospital, Glossop Road, Sheffield S10 2JF. 8179(A)

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Australian Telecommunications Commission Materials Analyst (\$A19,657-20,867*)

Telecom Australia invites applications from men and women for appointment to the above position in its Research Department located at Headquarters in Clayton, Melbourne, Victoria.

The successful applicant will be required to plan, supervise and participate in advanced chemical research and investigation into materials and develop methods for their application within Telecom Australia. The duties include development and application of complex analytical equipment and advanced methodology utilising Scanning Electron Microscopy (SEM), Secondary Ion Mass Spectrometry (SIMS), emission absorption and thermal analysis.

Qualifications

A tertiary degree or diploma in an appropriate physical science together with extensive research and administrative experience and leadership ability.

Conditions of Employment

will be similar to those in the Australian Public Service.

*At present rate of exchange £1=\$A1.70.

Interviews

It is anticipated that a senior Scientist of Telecom, Australia will be in London on or about 27/28 November to interview suitable applicants.

Please send a full curriculum vitae together with day-time telephone number on which you can be contacted to: Recruitment Officer, Public Service Board, Australia House, Strand, London WC2B 4LA to arrive by the 10th November.

8176(A)

LINCOLN COLLEGE

New Zealand

CHAIR IN ENTOMOLOGY (Agricultural Zoology)

The Council of Lincoln College, University College of Agriculture, invites applications for the Chair in Entomology, resulting from the impending retirement of Professor R. A. Harrison.

Applicants should hold an advanced degree in Science or Agricultural Science and have an established record of research and/or teaching in some branch of agricultural zoology.

The successful applicant will be appointed as Head of Department and will be responsible to the College Principal for the administration of his Department.

The appointment will be made within the existing range of professorial salaries, NZ\$23,012 to NZ\$29,188 per annum, the commencing salary being determined by the College Council.

Expenses of appointment reimbursed up to specified limits. New Zealand Government Superannuation is available.

Further details and general Conditions of Appointment are obtainable from the Secretary General, Association of Commonwealth Universities (Appts), 36 Gordon Square, London WC1H 0PF, or from the Registrar of the University.

Applications close on January 15, 1979 8089(A)

UNIVERSITY OF SURREY RESEARCH OFFICER— CARBON FIBRES DEPARTMENT OF METALLURGY AND MATERIALS TECHNOLOGY

A post has become vacant in our Fibres and Composite Materials Research Group for work on an investigation of the surface characterisation of high modulus carbon fibres.

The principal method of investigation will be, thermal desorption of surface active groups and their qualitative identification and estimation by mass spectrometry. The work will be supervised by Dr W. Watt, F.R.S., and carried out in collaboration with Materials Department, Royal Aircraft Establishment.

The post will be suitable for a graduate physical chemist or physicist with interests in surfaces and solid state.

Preference will be given to candidates with a lively interest in practical work and an imaginative approach to interpretation.

The appointment will be for one year. Starting date is visualised as November 1, 1978 or as soon as possible thereafter. Salary will be on an appropriate point on the Research and Analogous Staff Scales Range £3,384 to £4,882 per annum.

Applications in the form of a curriculum vitae, including the names and addresses of two referees should be addressed to Professor J. E. Bailey, Department of Metallurgy and Materials Technology, University of Surrey, Guildford, Surrey GU2 5XH 8113(A)

UNIVERSITY OF ZAMBIA

Applications are invited for the following posts in the
SCHOOL OF MINES

- (a) CHIEF TECHNICIAN
IN METALLURGY DEPARTMENT
- (b) CHIEF TECHNICIAN
IN MINING ENGINEERING
DEPARTMENT

Higher National Certificate, plus considerable experience in workshops or laboratories associated with Metallurgy and/or Mineral Dressing. For post (b), applicants should have considerable experience in workshops or laboratories with Mining (Drilling Machines, rock mechanics and ventilation laboratories, drawing ability). Duties include supervision of laboratories, setting up and maintaining equipment.

Salary scale, K5,316 to K6,324 per annum (£1 sterling equals K1.57). The British Government may supplement salaries by £3,606 per annum (Sterling) for married appointees and £1,620 per annum (Sterling) for single appointees (reviewed annually and normally, free of all tax) and provide children's education allowances and holiday visit passages, family passages, superannuation and medical aid schemes; regular overseas leave, various allowances. Detailed applications (two copies) with curriculum vitae and naming three referees to be sent direct to Registrar, University of Zambia, P.O. Box 2379, Lusaka, Zambia by November 22, 1978. Applicants resident in the UK should also send one copy to Inter-University Council, 90/91 Tottenham Court Road, London W1P 0DT. Further details may be obtained from either address. 8154(A)

UNIVERSITY OF CAPE TOWN SENIOR LECTURER/ LECTURER IN BIOCHEMISTRY

Applications are invited for a post, vacant from February 1979. Appointment, according to qualifications and experience, will be made on the salary scale—Senior Lecturer R8,460 by 360 to 9,900 by 450 11,250 per annum, or Lecturer, R6,3 by 360 to 9,810 per annum. In both cases, the salary will be supplemented by a pensionable allowance of 15.5%.

Candidates with a strong interest and experience in the following field will receive preference: structural chemistry of proteins and/or protein-nucleic acid interaction, structure as function of chromosome protein-hormone-receptor interaction.

Applicants should submit curriculum vitae, stating present salary, research interests and publications when available. If appointed, and names and addresses of three referees. The post may also be filled on a contract basis for 2 to 3 years with the possibility of renewal or conversion into a permanent post. Applicants should state whether they wish to be considered for contract appointment or a permanent post.

Memoranda concerning the position and general conditions of service should be obtained from the Registrar, Room 10, University of Cape Town, Private Bag, Rondebosch, C.P. 7700, by whom applications must be received not later than November 30, 1978 8090(A)

UNIVERSITY OF GUELPH College of Biological Science DEPARTMENT OF NUTRITION

Applications are invited for a position of Assistant Professor Nutrition. Candidates should be training in experimental nutrition nutritional biochemistry. The successful applicant will participate in teaching the metabolic aspects of nutrition at the undergraduate and graduate levels, and will develop a research program on some aspect of the nutrition evaluation of food. Curriculum vitae and names of three referees should be sent to Dr H. S. Bayley, Chairman of Search Committee, Department of Nutrition, College of Biological Science, University of Guelph, Ontario Canada N1G 2W1 by January 1, 1979. Appointment is subject to final budgetary approval 8092(A)

UNIVERSITY OF GUELPH College of Biological Science DEPARTMENT OF NUTRITION

Applications are invited for a contractually limited position of Assistant Professor in Nutrition (2 year term). Candidates should have training in experimental nutrition or nutritional biochemistry. The successful applicant will participate in one of the research programs within the Department. Curriculum vitae and names of three referees should be sent to Dr H. S. Bayley, Chairman of Search Committee, Department of Nutrition, College of Biological Science, University of Guelph, Guelph, Canada N1G 2W1 by January 1, 1979. Appointment subject to final budgetary approval. 8095(A)

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THE UNIVERSITY OF LEEDS

UNIT FOR CANCER RESEARCH AND THE UNIVERSITY COMPUTING SERVICE

Applications are invited for a temporary fixed term post of POSTDOCTORAL RESEARCH FELLOW for work on a project under the supervision of Professor E. H. Cooper (Unit for Cancer Research) and Professor M. Wells (University Computing Service). The project, which is supported by the M.R.C., will involve the development of computer based systems for the handling and analysis of multivariate information used for the monitoring of cancer in man, and assistance in its application by clinicians. A Ph.D. degree or equivalent experience is required and it is essential that candidates should have experience in statistics and practical experience of computing. The post is for a fixed period of three years.

Starting salary in the range £3,883 to £5,219 on the LA Scale for Research and Analogous Staff (£3,883 to £6,555) (under review).

Application forms and further particulars may be obtained from the Registrar, The University, Leeds LS2 9JT, quoting reference number 89/2/D. Closing date for applications November 16, 1978. 8099(A)

Applications are invited for two permanent positions in the Laboratories of the

SWISS SERUM AND VACCINE INSTITUTE Berne, Switzerland VIROLOGIST

Candidates should have strong background in Tissue Culture and Virology. Areas of competence should include human diploid cell culture methodology, virus production and development.

BACTERIOLOGIST

Join a team engaged in the development and production of bacterial vaccines. Applicants should have university degree. Previous experience in immunology and preparation of vaccines would be essential.

We offer excellent benefits. Salary commensurate with experience. Send curriculum vitae, bibliography, and references to: The Personnel Manager, SWISS SERUM AND VACCINE INSTITUTE, BERNE, P.O. Box 2707, CH-3001 Berne, Switzerland. 8110(A)

BHP Australia



Director Central Research Laboratories

The Broken Hill Proprietary Co. Limited (BHP) is the largest company in Australia with annual sales of A\$2,374 million and employing some 59,000 people in a variety of activities, covering steel; minerals; oil and gas and manufacturing. These activities, which range through exploration, mining; extraction; transportation and fabrication, are supported by extensive research and development programmes.

As a result of the promotion of the present incumbent to an executive position in Head Office, a new Director is being sought for BHP Central Research Laboratories which are one of two corporate laboratories for the BHP Group. The Director will be responsible for the total research programme in raw materials and processes on behalf of the operations divisions of the BHP Group and for external organisations under contract. The research fields covered, include coal conversion; agglomeration; iron and steel-making; combustion; electrometallurgy and hydrometallurgy and analytical sciences.

The laboratories were established in 1956 and are located at Shortland N.S.W., adjacent to the University of Newcastle. They currently employ 160 people, of whom 55 are professionals. The laboratories are well equipped by world standards and are essentially self-contained with respect to services. Their annual budget is of the order of A\$3.5 million.

Applicants should have research experience and a proven record in management. Salary will be in the range A\$30,000-40,000 p.a. depending on experience and qualifications, and a company car is provided. The Company operates a generous superannuation scheme and reasonable relocation and removal expenses will be paid. Subsidised accommodation will be paid for up to six months on arrival in Australia.

Applications containing full personal particulars, including age, qualifications, experience, publications, telephone numbers and names of referees should be marked 'Confidential' and addressed to:-

Dr. R. G. Ward, General Manager Research and New Technology, The Broken Hill Proprietary Co. Ltd., P.O. Box 86A, Melbourne, Victoria, Australia.

Applications should reach the above address by 15th January, 1979.

8137(A)

EAST MALLING RESEARCH STATION A SOIL SCIENTIST

to initiate studies on the effects of modern orchard management practices on soil chemistry and physics.

Candidates should have a first or upper second class Honours degree or the equivalent in an appropriate subject and at least two years' relevant postgraduate research experience.

Appointment as Higher Scientific Officer (£4,101 to £5,558) or Senior Scientific Officer (£5,154 to £6,898), grade and starting salary depending on experience. At least four years' relevant postgraduate experience normally required for appointment as SSO. Non-contributory superannuation.

Further details and application form from Assistant to the Secretary, East Malling Research Station, East Malling, Maidstone, Kent, ME19 6BJ. 8178(A)

ROTHAMSTED EXPERIMENTAL STATION Harpenden, Herts. AL5 2JQ BIOLOGIST

in the Soil Microbiology Department to assist with applied aspects of work on the physiology of VA endophytes and the possibility of their pot culture in artificial media, and additionally, to investigate techniques for production, storage, and field application of inoculum. The appointment will be for 3 years.

Qualifications: Honours Degree or equivalent qualification in a biological subject with some practical experience in applied crop physiology. Manual dexterity and ability to construct simple apparatus would be desirable.

Appointment in grade of Scientific Officer (£2,839 to £4,415). Starting salary according to qualifications and experience. Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees and quoting Ref. 375 by November 17, 1978. Further details on request. 8173(A)

WELSH NATIONAL SCHOOL OF MEDICINE (University of Wales) DEPARTMENT OF MEDICAL MICROBIOLOGY RESEARCH OFFICER

required from November 1, 1978 for a C.R.C.-supported 2-year research project involving studies on human tumour cells in culture and properties of hybrid cells prepared from them. Previous experience or a degree in biology or biochemistry is desirable. Starting salary for suitably-qualified applicants up to £4,133 per annum on the scale for University Research and Analogous Staff Grade 1B.

Application forms available from the Registrar and Secretary, Welsh National School of Medicine, Heath Park, Cardiff (tel. No. Cardiff 755944 ext. 2073) quoting ref. No. B7/22, further information available from Professor Watkins (tel. No. Cardiff 755944 ext. 2168). 8132(A).

Environmental Science

An opportunity exists for a person, distinguished in some aspects of the physical environmental sciences, to join the Environmental Studies Section of our Planning Department at our London Headquarters. The Section is responsible for environmental policy formulation within the CEEB based on a sound understanding of scientific principles.

The Section consists of a small number of specialists in different scientific and engineering disciplines who collectively cover a broad spectrum of activities in the air, water, noise and nuclear fields. It is intended that the person appointed would take an interest in much of the scientific work of the Section as a whole, but specifically would study in depth some particular important environmental problem – possibly but not necessarily, the global CO₂ balance. In this he/she would need to collaborate with other experts in the CEEB, UK and throughout the world.

It is not envisaged that he/she would carry direct responsibility for the day-to-day activities of the Section. The post could well appeal to a senior University scientist of professional, or near, standing wishing to develop an understanding of the problems of a large organisation in caring for the environment in a responsible way. Equally it could appeal to an experienced industrial scientist wishing to broaden his/her experience. The appointment would be for a fixed term, the length of which might be up to three years. This aspect will be discussed with candidates called for interview.

The salary will reflect the seniority of the post and is unlikely to be less than £9000 p.a. and could be up to £11,000 p.a.

Applications stating full relevant details and present salary to the Group Personnel Officer, CEEB, Sudbury House, 15 Newgate Street, London EC1A 7AU, by 2 November. Quote Ref. NS/2103.

CENTRAL ELECTRICITY GENERATING BOARD HEADQUARTERS

6079(A)



UNIVERSITY OF ST ANDREWS DEPARTMENT OF CHEMISTRY

Applications are invited for a post of Postdoctoral Fellow to work on a project concerned with methods of modifying carbon fibre. The project is supported by the Ministry of Defence and will be run in close liaison with the Royal Aircraft Establishment, Farnborough.

The post is for one year in the first instance at a salary within the range £3,883 to £4,382 per annum plus U.S.S.

Applications, with the names of three referees, should be sent as soon as possible to Dr J. R. MacCallum, Department of Chemistry, Purdie Building, The University, St Andrews, Fife KY16 9ST, from whom further particulars may be obtained. 8116(A)

EUROPEAN MOLECULAR BIOLOGY LABORATORY E.M.B.L.

TECHNICAL ASSISTANT

Applications are invited for the post of technical assistant in the field of electron microscopy of nucleic acids in Dr Delius' research group.

Appropriate technical training together with knowledge of more than one of the official languages of the Laboratory (French, English, German) are required.

The salary offered will be between DM2,100 and DM2,600 monthly. Certain allowances are payable according to personal circumstances.

Please write for an application form, quoting reference no. 78/34 to:

E.M.B.L.,
Personnel Section,
Postfach 10.2209,
D-6900 Heidelberg,
Germany.

8170(A)

MAX-PLANCK-INSTITUT WEST BERLIN There is a vacancy for a PROTEIN CRYSTALLOGRAPHER

to work on the crystallisation and structure analysis of ribosomal proteins. Applicants should have experience in the analysis of protein structures.

The position is available from January 1979 and is initially for three years with the possibility of renewal.

Salary will be in the range of DM40,000 to DM50,000 per year.

Applications are invited giving information about qualifications and the addresses of 2-3 referees. They should be sent to Professor H. G. Wittmann, Max-Planck-Institut für Molekulare Genetik, Ihnstrasse 63-73, D-1000 Berlin 33, Germany. 8175(A)

PHYSICAL OCEANOGRAPHER

to fill vacant faculty post. Level of appointment commensurate with experience and qualifications. Applicant should have experience in the planning and execution of scientific cruises. Experience in one or more of the large, international oceanographic field programs desirable. Duties include teaching graduate level courses in physical oceanography and supervising the research of graduate students. Interested applicants should send curriculum vitae, including bibliography and two references, by November 30, 1978 to: Professor J. E. Gentry, Chairman, Division of Meteorology and Physical Oceanography, Rosenstiel School of Marine and Atmospheric Science, University of Miami, 4600 Rickenbacker Causeway, Miami, FL 33149.

The University of Miami is an Affirmative Action/Equal Opportunity Employer. 8093(A)

UNIVERSITY OF GLASGOW

DEPARTMENT OF CLINICAL ONCOLOGY RESEARCH ASSISTANT and POSTDOCTORAL RESEARCH FELLOW

Applications are invited for the posts of Research Assistant and Postdoctoral Research Fellow, tenable for up to three years, for research on anti-metastatic drugs in animal tumour studies.

The Research Assistant should possess a good honours degree in a bioscience subject and will be expected to register for a higher degree. Starting salary £3,384 per annum.

The Research Fellow should possess experience in working with small animals and have recently obtained a Ph.D. degree in a bioscience subject. Starting salary £4,382 per annum.

Applications, together with two copies of a Curriculum Vitae and the names of two referees, should be sent to: Professor K. C. Calman, Department of Clinical Oncology, 1 Horsehill Road, Glasgow G12 9LY. 8155(A)

UNIVERSITY OF SURREY CHAIR IN PATHOLOGY

Applications are invited for this new Chair in Pathology in the Department of Biochemistry of the University, which is to be held in collaboration with the Medical Research Council Toxicology Unit, Carshalton, Surrey. Suitable candidates who would be medical or veterinary pathologists of considerable experience would be expected to contribute to existing teaching and research programmes in Toxicology and to develop new post-graduate activities in experimental and comparative Pathology.

An Honorary Consultant appointment with the South West Thames Regional Health Authority may be arranged for an appropriately qualified candidate and facilities in Human Pathology will be available within the local Guildford Hospital.

Salary will not be less than £9,443 per annum.

Further particulars may be obtained from the Academic Registrar (L.F.G.) University of Surrey, Guildford, Surrey GU2 5XH or telephone Guildford 71281, ext. 452. Applications from men and women in the form of a curriculum vitae including the names and addresses of three referees should be sent to the above address by November 24, 1978. 8159(A)

UNIVERSITY OF LIVERPOOL

DEPARTMENT OF BOTANY Mineral Industries Research Organisation Research Project

Applications are invited for two posts of
SENIOR
RESEARCH ASSISTANT

to develop guidelines for the mineral extraction industry for the establishment of vegetation on (A) metalliferous wastes, (B) disused quarries, by field experimentation and literature survey. Applicants should have research experience in plant ecology, soil science or geochemistry, and be interested in working in collaboration with industry.

The project is financed for a period of two years. The initial salary will be either £3,883 or £4,133 per annum.

Applications, together with the names of two referees, should be received not later than November 17, 1978, by The Registrar, The University, P.O. Box 147, Liverpool L69 3BX, from whom further particulars may be obtained. Quote Ref: RV/974/N/A or B. 8166(A)

MEDICAL RESEARCH COUNCIL

National Institute for Medical Research DIVISION OF MICROBIOLOGY

There is a vacancy for a
POSTDOCTORAL SCIENTIST

to join a small group studying genetic and molecular aspects of the replication and function of nuclear and mitochondrial genomes in yeast. Areas of interest include repair, replication and recombination of DNA in relation to both the vegetative cell cycle and meiosis, and current projects include studies on conditional DNA synthesis and cell cycle mutants.

The appointment will be for up to three years and will be in the salary range £4,655 to £6,080 per annum according to age and qualifications plus £450 p.a. London Allowance-Superannuation provision.

Applications, giving details of qualifications, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA, before November 30, 1978, quoting reference M1. 8097(A)

CHANGE OF ADDRESS

The Advertisement offices for NATURE have moved to
3 DYERS BUILDINGS, LONDON, EC1N 2NR
TELEPHONE: 01 831-6901



University of Wales

WELSH SCHOOL OF PHARMACY

A. PROFESSOR PHARMACEUTICS

research interests preferably in Pharmaceutical Technology

LECTURERS (2)

B. PHARMACOGNOSY

interests in analytical work

C. PHARMACOLOGY

Physiology and Pharmacology, with research interests in either neuropharmacology, cardiovascular pharmacology or drug metabolism and kinetics

Salaries:

Post A Professorial Range from £9,820

Posts B and C £3,883 to £7,754 (Interim range and scale)

Requests (quoting Post and Ref: N) for details and application form, to

Personnel Section (Academic), UWIST, Cardiff CF1 3NU

Closing date 24 November, 1978

8082(A)



Wellcome

Helminthologist

Department of Parasitology

(£6,000 – £10,000)*

Applications are invited for the post of Section Leader of Helminth Chemotherapy in the Department of Parasitology (Head, Dr. R. A. Neal) of the Wellcome Research Laboratories located in Beckenham, Kent. The department is concerned with research for the control of selected veterinary and medical parasitic diseases by chemotherapeutic and immunological methods. There is a scientific staff of 16 covering biochemical, chemotherapeutic and immunological aspects of protozoal and helminthic infections.

The helminthologist appointed will be required to assume responsibility for chemotherapeutic studies covering a broad range of parasites and to initiate original research with particular emphasis on nematodes. Part of the work will involve collaboration within a multidisciplinary team.

Applicants should have substantial relevant experience and proven ability for original research. Interest in the biochemistry of nematodes would be advantageous but not essential. Candidates with biological, veterinary or medical backgrounds would be considered.

The Wellcome Research Laboratories are located in pleasant parkland surroundings 12 miles south of the centre of London. *Salary, dependent on ability and experience, will be in the range of £6,000 – £10,000. Fringe benefits are excellent and include generous relocation expenses where appropriate.

Please write, giving brief details of qualifications and experience, to the Personnel Manager, The Wellcome Research Laboratories, Langley Court, Beckenham, Kent BR3 3BS.



8188(A)

MASSEY UNIVERSITY

Palmerston North, New Zealand

LECTURER IN ENVIRONMENTAL HORTICULTURE

Applications are invited for the above-mentioned position in the Department of Horticulture and Plant Health, the Faculty of Agricultural and Horticultural Sciences.

The appointee will be required to assist with the teaching, at degree and diploma levels, all courses in the development and maintenance of ornamental plantings. Practical experience in these areas together with a strong background on plants and their use in the environment is required. An interest in landscape planning, design and construction would be desirable.

Commencing salary, according to qualifications and experience, within the range NZ\$11,894 to NZ\$14,488.

Further details of the position, and of the University together with the conditions of Appointment may be obtained from the Association of Commonwealth Universities (Acpu), 36 London Square, London WC1H 0PF, or the Registrar of the University. Applications close on December 2, 1978.

8183(A)

LECTURER IN CIVIL ENGINEERING

CONTINUUM MECHANICS

A vacancy exists in the Department of Civil Engineering for a Lecturer with a background in Theoretical Continuum Mechanics and a productive research interest in this field relevant to Civil Engineering.

The Department has well established analytical and experimental groups working in this area and the person appointed would, therefore, be able to collaborate immediately with active colleagues.

In addition, he/she would be required to teach Mechanics in the Department at both undergraduate and postgraduate levels.

Salary on scale £3,884 to £7,754 (under review) according to qualifications and experience.

Further particulars may be obtained from D. A. S. Copland, The University, Southampton SO9 5NH to whom applications (seven copies from UK applicants) should be sent by November 30, 1978. Please quote reference number 168/A/Na. 8084(A)

HEAD OF PHARMACOLOGY DEPARTMENT

A leading Spanish Pharmaceutical Company situated in the Mediterranean city of Barcelona invites applications from pharmacologists for the above post in a Pharmacology Department. The man we appoint will have a good honours degree in an appropriate discipline, a Ph.D. in pharmacology and several years postdoctoral experience preferably in the pharmaceutical industry.

The successful applicant will be responsible to the director of Biological Sciences and must be able to formulate and organise research and development projects and collaborate with the other departments in the Biological and Chemical science groups.

Knowledge of Spanish is not initially essential but the successful candidate must be prepared to acquire adequate fluency in this language within 2 years. The salary is tax free and negotiable at around 30,000 dollars p.a. and special assistance will be given for removal expenses to Barcelona.

Applications in writing with full curriculum vitae should be addressed to:

Pharmacology Department
P.O.B. 1583
BARCELONA-24 (Spain)

8189(A)

CHANGE OF ADDRESS

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TELEPHONE : 01 831-6901

**MAFF: Agricultural Science
Service Regional Pests Organisation**

Biologists

■ Advise farmers, land owners, local authorities, food manufacturers and importers on the control of vertebrate pests and assist with training and lecturing ■ Co-operate with Pest Infestation Control Laboratory in research, field trials and surveys.

Locations

□ Preston, Ruthin, Cardiff, Bristol, Norwich and Brigg (nr Scunthorpe) □ Vacancies may arise elsewhere.

□ Good honours degree in a biological/agricultural subject □ Appropriate practical experience preferred □ Age normally under 27 □ Appointment as Scientific Officer (£2840-£4410 depending on qualifications and experience) □ Ref: SB/3/AL.

■ Application forms (for return by 17 November 1978) from Civil Service Commission, Alencon Link, Basingstoke, Hants, RG21 1JB, telephone Basingstoke (0256) 68551 (answering service if office closed).

**Science
Group**
CIVIL SERVICE

8187(A)

MERCK SHARP & DOHME-CHIBRET

Subsidiary of MERCK and Co
develops an International Research Center
located in the Herat of France at RIOM (Puy de Dôme)
and SEEKS for its
SAFETY ASSESSMENT DEPARTMENT

veterinary pathologists toxicologists teratologists

Applicants with a DVM, PhD or equivalent and adequate post-doctoral training should have experience and relevant publications in Laboratory Animals.

A professional environment is offered in staff, equipment and facilities. Candidates should have French as their conversational language and a good working knowledge of English. A training period in the U.S. has to be considered.

Hand written applications with a detailed curriculum vitae and expectations should be addressed to:

MSD
MERCK
SHARP
DOHME

Direction du Personnel
200, Boulevard E. Clémentel
63018 CLERMONT FERRAND Cedex - France

8171(A)

THE UNIVERSITY OF LEEDS

DEPARTMENT OF BIOCHEMISTRY

Applications are invited for the temporary post of

DEMONSTRATOR

in the above Department from suitably qualified candidates. The person appointed will participate in teaching biochemistry to science and to medical students and will also be expected to carry out research on the molecular biology of bacterial drug resistance in collaboration with Dr J. H. Parish. The post is tenable from January 1, 1979 until September 30, 1979. Salary at an appropriate point on the IB Scale for Other Related Staff (£3,384 to £5,604) (under review) according to age, qualifications and experience.

Application forms and further particulars may be obtained from the Registrar, The University of Leeds, Leeds LS2 9JT, quoting reference number 83/16/D. Closing date for applications November 6, 1978. 8002(A)

PHYSIOLOGIST

Ph.D. needed in active Hematology Laboratory, Thomas Jefferson University, Philadelphia, Pa., with experience in measurements of platelet size, morphology, and functions related to the evaluation of patients and platelet transfusion. Minimum salary, \$15,000 plus commensurate with experience. Excellent employee benefits. Send detailed resume with salary requirements to Mr Burvin Hardy, Bureau of Employment Security, 1709 S. Broad Street, Philadelphia, Pa. 19148. Affirmative Action/Equal Opportunity Employer. 8119(A)

UNIVERSITY OF MIAMI ASSISTANT/ASSOCIATE PROFESSOR

DEPARTMENT OF BIOLOGY

Three new positions available 1979 in research-oriented department emphasising organism-level studies.

1. **TROPICAL FOREST BIOLOGIST.** Botanist with research interests in community ecology or forest structure preferred. Appropriate areas can include succession dynamics, seed dispersal, competition, plant-animal interactions, co-evolution, phenology, etc.

Preference for the following positions given to candidates with a demonstrated interest in tropical organisms.

2. **VERTEBRATE PHYSIOLOGIST.** Interdisciplinary research in behaviour, neurobiology, or ecology preferred.

3. **EVOLUTIONARY BIOLOGIST/POPULATION GENETICIST.** Specific interests might include genetic structure of populations, dynamics of natural selection, reproductive life history strategies, etc.

Candidates must have Ph.D. or equivalent and an active research program. Competitive salary based on qualifications and experience. Apply before January 10, with curriculum vitae, a recent reprint, and names of four references (have overseas letters of recommendation sent immediately) to: Search Committee for [position sought], Department of Biology, Box 249118, University of Miami, Coral Gables, Florida 33124, USA. Equal Opportunity Employer. 8121(A)

UNIVERSITY OF GLASGOW SECOND CHAIR OF PSYCHOLOGY

Applications are invited from those with experience and interest in any area of Psychology for a Second Chair which will be established on October 1, 1979.

Further particulars may be had from the Secretary of the University Court (Room 18), The University of Glasgow, Glasgow G12 8QQ, to whom applications (15 copies) giving the names and addresses of three referees, should be lodged on or before December 12, 1978.

In reply please quote Ref. No. 4265M. 8102(A)

HUMAN GENETICIST

To teach human and general genetic and to conduct research in human genetics, Assistant or Associate Professor level. Apply with curriculum vitae, including research interests and names of three references. Position open through February 28, 1979. Write to:

James B. Boyd
Department of Genetics
University of California
Davis, California 95616

The University of California is an equal opportunity and affirmative action employer and invites applications from all qualified individuals. 8103(A)

MINISTRY OF OVERSEAS DEVELOPMENT

TROPICAL PRODUCTS

INSTITUTE

CULHAM, OXON.

MECHANICAL ENGINEER

The above Research Institute, which carries out scientific research to aid developing countries, invites applications for a Mechanical Engineer to work in the Cereals Technology Section, of its Industrial Development Department at Culham. The appointee will assist in the development of machines for the milling and debranning of cereal grains. They will also be required to carry projects through from inception, design and construction to the testing stage. Experience of the type of work is not necessary but the applicant should possess a sound practical understanding of engineering techniques. Short-term overseas visits could be involved. This is a permanent appointment of two years, grade Scientific Officer; Salary: £2,839 + £4,415; Conditions of Service: 5-day week of 42-hours (including lunch breaks); 20 days leave per year; not contributory pension scheme; Qualifications: 1st or 2nd Class Honours degree in Mechanical Engineering.

Application forms from Miss C. A. Hall, Tropical Products Institute, 12 Clerkenwell Road, London EC1R 5DF. 8104(A)

LECTURER IN PHARMACOLOGY

This new post arises from the expansion of the Department of Pharmacology with the appointment of Professor H. P. Rang as Chairman.

The person appointed will be involved in teaching medical, dental and science students and will be expected to pursue an active research programme.

Closing date for applications: December 1, 1978. Further particulars from Professor J. L. Mongar (N. Department of Pharmacology, University College London, Gower Street WC1E 6BT. 8088(A)

CHANGE OF ADDRESS

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3 DYERS BUILDINGS, LONDON, EC1N 2NR
TELEPHONE: 01 831-6901

SENIOR RESEARCH FELLOW IN ATOMIC/MOLECULAR PHYSICS

The University of Maryland and the National Bureau of Standards in Washington, D.C. are announcing a one year fellowship for a senior research scientist to pursue research at the N.B.S. Synchrotron Ultraviolet Research Facility (S.U.R.F.). The S.U.R.F. fellow will hold an appointment in the Institute of Physical Science and Technology at the College Park campus of the University of Maryland. In addition to his research duties at S.U.R.F., the appointee will interact with the faculty and students at the University, principally through lectures and seminars.

Applications are now being accepted for the academic year '79-80, with detailed schedule to be negotiated. Salary and faculty rank will be commensurate with current position. We are initially seeking an individual interested in pursuing atomic/molecular physics at S.U.R.F. Experience in the use of synchrotron radiation and/or photoelectron spectroscopy of gases will be considered particularly valuable for the appointment.

Send résumé, desired starting date, and research plans to Professor Joseph Silverman, Institute for Physical Science and Technology, University of Maryland, College Park, Maryland 20742 8122(A).

TECHNICIAN

Required at our Mill Hill Laboratories, London NW7, to join group working on experimental embryology and regeneration. Work will involve some animal maintenance (amphibia), histology, photography, laboratory maintenance and experimental work.

H.N.C./Degree Laboratory experience an advantage. Salary range 3,261 to £4,902 L.W. Application form from Mrs Nelson, Imperial Cancer Research Fund, Lincoln's Inn Fields, WC2, Tel. 242 0200 ext 305, quoting ref 13/79. Closing date for applications November 7, 1978 8033(A).

INSTITUTE OF CANCER RESEARCH SUTTON, SURREY DIVISION OF EPIDEMIOLOGY SCIENTIST FOR COMPUTER SIMULATION

A health-hazard monitoring system in industrial situations. A small research team will undertake this project, to study design and analysis of such an information system using simulation techniques. Applicants should preferably have a degree in mathematics or statistics, probably an M.Sc. or experience relevant to mathematical modelling of medical statistics. The post is for two years, with salary in the range £3,909 per annum to £4,408 per annum plus London allowance of £450 per annum, as an R.C. Grade II scientist. Further details available from Mrs Bell (01-643 8901, ext 291). Applications in duplicate with the names of two referees to the Secretary, Institute of Cancer Research, 34 Cancer Place, London SW7 3NT, quoting ref 300/G/134. Closing date for applications November 10 1978 8138(A).



Wellcome Clinical Research Associate—Vaccines and Sera

The Wellcome Foundation has been a pioneer in the development of vaccines and sera for immunisation against infectious disease for over 80 years and has made many original scientific contributions in this field.

We currently have an opportunity for a Clinical Research Associate in the Department of Clinical Immunology & Chemotherapy of our Research Laboratories located in Beckenham, Kent. The successful candidate's main duties will include

- the identification and recording of scientific and clinical reports related to the Foundation's interest in vaccines and sera,
- the preparation of authoritative and critical scientific reviews,
- participation in reviews of clinical research projects,
- dealing with some technical queries;
- assisting in the administration of clinical trials/data handling in association with the responsible physician.

Candidates should have a degree in a biological science and some years postgraduate experience in a relevant field e.g. virology, bacteriology or immunology. A knowledge of immunological theory and the nature, clinical characteristics and usage of vaccines would be particularly advantageous.

The Laboratories are situated in pleasant parkland surroundings some 12 miles south of the centre of London. Starting salary will depend upon age and experience but is not expected to be less than £4,250 p.a. Conditions of employment are excellent and include generous relocation expenses where appropriate, pension scheme, subsidised canteen and sports and social facilities.

For an application form, please write to:—

Personnel Manager,
Wellcome Research Laboratories,
Langley Court,
Beckenham, Kent.
BR3 3BS.



8184(A)

ONLINE TRAINING OFFICER

To conduct training sessions with Commonwealth Agricultural Bureaux staff and customers, and organise exhibits and displays. Subjects covered include all of C.A.B.'s products and services, particularly online information retrieval.

Applicants should be graduates in agricultural science or related subject. You must be a car owner, with a clean driving licence. Although based at Farnham Royal the position demands considerable travel in the UK and overseas for which allowances will be paid.

A knowledge of one or more European languages would be advantageous.

Salary in the scale £2,839 to £5,448 per annum Superannuation 22 days' annual leave.

Apply to Sales Director, Commonwealth Agricultural Bureaux, Farnham House, Farnham Royal Slough SL2 3BN 8151(A).

UNIVERSITY APPOINTMENTS IN NEW NIGERIAN UNIVERSITIES

The Inter-University Council intends to extend its register of candidates available for posts in the new and developing universities in Nigeria. Applicants should possess a good Honours Degree and research experience preferably with a Doctorate. University level teaching experience is desirable for Lectureship posts and essential for Senior Lectureships. The universities in question are often developing faster than the supporting infrastructure and a pioneering approach is essential. Applicants on the basis of possible secondment from a British university/polytechnic would be welcomed.

Applicants are invited for posts of Lecturer and Senior Lecturer in the following subjects:

- | | |
|----------------|---------------------------|
| B1 MATHEMATICS | B6 GEOLOGY |
| B2 PHYSICS | B7 MECHANICAL ENGINEERING |
| B3 CHEMISTRY | B8 ELECTRICAL ENGINEERING |
| B4 BOTANY | B9 CIVIL ENGINEERING |
| B5 ZOOLOGY | |

Salary range £4,350 p.a. to £6,905 p.a. plus 25 per cent Contract Bonus. Salary may be supplemented in appropriate cases for posts at Senior Lectureship level.

Appointments normally on contract for 2/3 years renewable. Paid overseas passages every 2 years, car loan, car allowance, furnished accommodation at low rental as available.

Further information and form from Division A, Inter-University Council, 90-91 Tottenham Court Road, London W1P 0DT. Closing date December 1, 1978.

Candidates living outside the United Kingdom should not apply to the I.U.C. but to the universities in Nigeria directly. The universities concerned are those at Calabar, Ilorin, Jos, Kano, Maiduguri, Port Harcourt, Sokoto 8180(A).

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TELEPHONE : 01 831-6901

Gallagher LIMITED

CHEMIST FOR BIOLOGICAL RESEARCH

Gallagher Limited R. & D. Division (Belfast) require a graduate as leading member of a team working on structure-activity correlations of tobacco smoke components. The successful applicant will be a chemist or biochemist aged 25-35 with relevant experience in academic or industrial research. Practical experience should include chromatographic separation techniques. This is a demanding post; the holder of which will need to display marked creativity and leadership.

The laboratories are well equipped; working conditions are pleasant and include a 36½-hour 4½-day week. Salary will fully match the importance of the post and the experience of the candidate.

Applications to Dr. R. R. Boxall
GALLAHER LIMITED
Henry Street, Belfast BT15 1JE

8161(A)



DORSET AREA HEALTH AUTHORITY
EAST DORSET HEALTH CARE DISTRICT

RESEARCH TECHNICIAN

IMMUNOLOGY DEPARTMENT

at ROYAL VICTORIA HOSPITAL
BOURNEMOUTH

A temporary research assistant medical laboratory scientific officer is required for the Department of Pathology in Immunology at the above Hospital. The post is for approximately a five months period only and is to assist in the Department's Regionally Financed Programme. Knowledge of column chromatography and radio-immune assay is essential.

Normal working hours will be 9.00 a.m. to 5.30 p.m. Monday to Friday inclusive.

Closing date for receipt of applications 9th November 1978

Application forms and job descriptions are obtainable from Miss M. Smith, Staff Appointments Officer, Royal Victoria Hospital, Shelley Road, Bournemouth BH1 4JG. Tel: 0202 35201 ext. 381 (between 9.00 a.m. and 4.00 p.m. Monday to Friday only). 8101(A)

DALHOUSIE UNIVERSITY DIRECTOR OF ENGINEERING PHYSICS PROGRAMME

Applications are invited for the position of Director of the programme in Engineering Physics within the Department of Physics, Faculty of Arts and Science. Candidates should have a doctoral degree in Engineering Physics or closely allied field, be eligible for registration as a professional engineer and have suitable industrial experience and inventive ability. Teaching involves both undergraduate and graduate students. Appointment at the associate or full professor level, with salary according to qualifications and experience, commencing July 1, or September 1, 1979. Canadian citizens and landed immigrants are particularly urged to apply. Enquiries or applications should be sent as soon as possible to The Dean, Faculty of Arts and Science, Dalhousie University, Halifax, Nova Scotia, Canada B3H 3J5. 8111(A)

ST THOMAS'S HOSPITAL MEDICAL SCHOOL (University of London) London SE1 7EH

Applications are invited from medically or scientifically qualified persons for the post of

RESEARCH ASSOCIATE

in the Department of Chemical Pathology and Metabolic Disorders. The successful candidate will work with Professor B. Lewis and Dr N. Miller on cholesterol and lipoprotein metabolism in tissue culture on a topic which would be suitable for a doctoral thesis. Preference will be given to applicants with previous research experience in cell biology, but such experience is not essential. Salary on University scale for Research staff, plus London Allowance. Further information from Professor Barry Lewis (Tel: 01-928 9292 ext. 2264) to whom applications (3 copies with the names of 2 referees) should be forwarded by November 25, 1978. 8115(A)

NEUROANATOMIST THE DEPARTMENT OF PHYSIOLOGY-ANATOMY UNIVERSITY OF CALIFORNIA, BERKELEY

invites applications and nominations for the position of Assistant Professor of Anatomy which will become available July 1, 1979, pending final budgetary approval. The successful candidate must be able to assume primary teaching responsibility for one undergraduate and one graduate neuroanatomy course in alternate years, and to participate in the Department's teaching program in the anatomical sciences. Preference will be given to applicants with two or more years of postdoctoral training and research competence in developmental and/or functional anatomy of the nervous system. Candidates will be evaluated on the basis of their dedication to excellence in teaching and research, and their capacities to develop a vigorous and independent research program in neurobiology. Interested individuals should reply by letter, explaining their qualifications, with a current curriculum vitae and a list of three or more persons who could act as referees, to: Search Committee, Department of Physiology-Anatomy, 2549 Life Sciences Building, University of California, Berkeley, California, 94720. Deadline for applications and nominations: January 1, 1979. The University of California is an affirmative Action Employer. 8117(A)

LANCHESTER POLYTECHNIC AT COVENTRY DEPARTMENT OF BIOLOGICAL SCIENCES POSTDOCTORAL RESEARCH FELLOW

Salary within the range: £4,101 to £6,558 (it is only possible to appoint on the minimum of this scale). Tenable for three years, from January 1, 1979.

RESEARCH TECHNICIAN

Salary £3,279 to £4,146 T2/3. Tenable for three years from January 1, 1979.

To participate in a joint project S.R.C. and I.C.D. involving development of a method for the *in vitro* culture of perfused hepatocytes supported in a 3 dimensional matrix as an application to toxicological studies. Applicants for both posts should offer experience in animal cell culture and in biochemistry. Knowledge of cytological procedures would also be an advantage.

Applicants for the Research Technician post should possess an H.N., H.N.D. or degree in an appropriate subject.

- * Good working conditions.
- * City centre site.
- * Refectory facilities.

For application forms and further particulars please apply in writing enclosing a foolscap stamped addressed envelope to the Personnel Officer, Lanchester Polytechnic, Priory Street, Coventry. Applicants in contact Dr. Sturdee or Dr. Cris informally by telephoning the Polytechnic (Coventry 24166).

Closing date for applications: November 13, 1978. 8123(A)

UNIVERSITY OF OXFORD DEPARTMENT OF PHYSICS

CLARENDON LABORATORY

A post is available to work as Postdoctoral Research Officer in a group concerned with the chemical and physical principles of wear during machining with diamond and other materials. The post, which is for period of two years, involves a limit amount of demonstrating, and requires an interest in both physical and chemical principles and their application to engineering practice. Scale £3,384 to £4,466 (under review starting point, according to age, e.g. £3,883 at age 24).

Application, with curriculum vitae and names of two referees, should be sent to Dr J. Wilks, Clarendon Laboratory, Oxford, OX1 3PU, from whom further details may be obtained. 8139(A)

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF GEOGRAPHY LECTURESHIP

Applications are invited for the post of Lecturer in Urban Geography. Preference may be given to candidates with mathematical or statistical interests.

Salary will be in the range £3,800 to £7,754, plus superannuation. Maximum starting salary will be according to age, experience and qualifications.

Further particulars are available from the Assistant Registrar, Science and Engineering, University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (the copies) including full curriculum vitae and naming three referees, should be sent by Monday, November 27, 1979. Please quote ref. NH4. 8124(A)

CHANGE OF ADDRESS

The Advertisement offices for NATURE have moved to
3 DYERS BUILDINGS, LONDON, EC1N 2NR
TELEPHONE: 01 831-6901

THE QUEEN'S
UNIVERSITY OF
BELFAST
LECTURESHIP IN
BIOCHEMICAL
PHARMACOLOGY

Applications are invited from persons holding a university degree, and having experience and training in Biochemical Pharmacology, or Pharmacology for a lectureship in Biochemical Pharmacology in the Department of Therapeutics and Pharmacology tenable from April 1, 1979, or such other date as may be arranged. Duties will include teaching pharmacology to medical, dental and postgraduate students, and contributing to research in biochemical pharmacology. Experience in pharmacokinetics and in measurement of plasma levels of drugs is highly desirable and an interest in pharmacology and/or clinical pharmacology would be an advantage.

Initial placing which will depend on experience and qualifications will be made on the Scale (under review) £3,885 to £7,755 with contributory pension rights under the F.S.S.U./U.S.S. Assistance is available with removal expenses.

Further particulars may be obtained from the Personnel Officer, The Queen's University of Belfast, BT7 1NN, Northern Ireland. Closing date: November 30, 1978. (Please quote Ref: 78/N).

8185(A)

COMMONWEALTH
AGRICULTURAL BUREAUX
Vacancy for
SCIENTIFIC INFORMATION
OFFICER

at the

COMMONWEALTH BUREAU
OF ANIMAL HEALTH

Central Veterinary Laboratory,
New Haw, Weybridge, Surrey

General Information. The work of the Bureau is to collect and make available through its publications the *Veterinary Bulletin* and *Index Veterinarius* and by magnetic tape services, information relating to all aspects of veterinary science.

Main duties. The duties include archiving scientific periodicals, indexing articles, preparing English-language abstracts for the monthly journal *The Veterinary Bulletin*, editorial work, dealing with enquiries and preparing reviews and bibliographies.

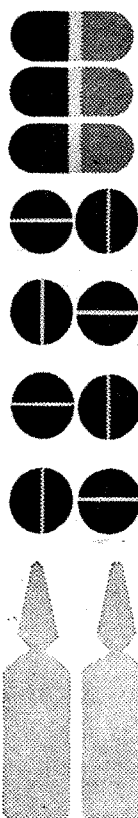
Qualifications. A qualification in information science or an appropriate biological science is required, plus a knowledge of European and Slavonic languages or a willingness to learn languages.

Salary. In the scale £2,839 to £5,448 per annum plus an Outer London weighting allowance of £275 per annum, plus a compensatory allowance taxable but not superannuable) of 3% to offset personal contribution.

Superannuation Scheme—part-time appointment *pro rata*. Starting salary according to qualifications, experience and age.

Application forms and full particulars on the Executive Director, Commonwealth Agricultural Bureaux, Pirbright House, Pirbright, Woking, Surrey, SL2 3BN, to whom completed applications (in duplicate) should be sent by November 27, 1978.

8107(A)



Laboratory Head— Biochemistry New Fermentation Products

The Glaxo Group of companies has an impressive history of exploiting fermentation products. Our Fermentation Research Division, situated in pleasant surroundings at Stoke Poges in South Buckinghamshire, has an extensive programme to investigate the biosynthetic potential of a variety of micro-organisms.

We are seeking an experienced research microbiologist, with a good innovative record, capable of supervising a graduate and technical staff and able to liaise with biochemists, chemists and fermentation technologists within a progressive multi-disciplinary research group.

The successful candidate will be responsible for organising and reporting the work of a laboratory and should combine an understanding of microbial metabolism and the optimisation of secondary metabolites in liquid culture with a capacity for a critical evaluation of the potential of selected organisms for new product formation.

Starting salary will be in the range of £5,320 p.a. to £7,073 p.a. subject to qualifications and experience. In addition there is a London Allowance of £258.50 p.a. and pension and bonus schemes. Assistance with relocation expenses will be given where appropriate.

Please apply to:

Mr. R. E. Nolan
Personnel Officer
Glaxo Allenburys Research (Greenford) Ltd.
Greenford Road,
Greenford
Middx.

Quoting reference number: 247

8186(A)

Glaxo-Allenburys Research (Greenford) Limited

LABORATORY TECHNICIAN for duties including assisting research staff and helping run student practical classes. Applicants should possess H.N.C. or equivalent qualification and relevant experience. Salary on Whitley Council Scales depending upon qualifications and experience. Applications in writing with names of two referees to: Mr S. W. Phillpot, Department of Physiology, The Middlesex Hospital Medical School, Windeyer Building, Cleveland Street, London W1P 7PN.

8086(A)

UNIVERSITY OF LONDON
KING'S COLLEGE
DEPARTMENT OF
PHYSIOLOGY

Applications are invited for a newly created Lectureship in Physiology. The Department of Physiology is engaged in teaching medical, dental and science students and has good facilities for research. Applicants should preferably have an interest in and be able to teach Mammalian Physiology. Candidates active in inter-disciplinary fields such as genetic, developmental or behavioural physiology will not be excluded. A medical qualification although desirable is not essential.

Salary scale £3,883 to £7,754 per annum plus £450 per annum London Allowance. The starting salary will be at an appropriate point on the scale. Universities Superannuation Scheme contributions would be payable.

Application forms and further particulars are available from the Assistant Registrar, King's College London, Strand, London WC2R 2LS and should be returned with any additional information, in triplicate, to reach him not later than Friday, December 1, 1978 quoting reference 191647/N.

8087(A)

UNIVERSITY OF
NEWCASTLE UPON TYNE
SCHOOL OF PHYSICS

Applications are invited for the post of Research Associate to continue studies in Lunar Magnetism and Seismology.

The School of Physics has a programme for the study of the magnetism of lunar rocks and is concerned over the theoretical consequences of early lunar magnetic field for the understanding of the evolution of the interior of the moon and its present internal activity.

The appointment will be for one year only, at a salary of £4,882 plus F.S.S.U. and National Insurance, and will be made from a mutually agreed date.

Applications (two copies) together with names and addresses of two referees should be sent to Professor S. K. Runcorn, School of Physics, The University, Newcastle upon Tyne NE1 7RU, as soon as possible.

8112(A)

THE UNIVERSITY of Leeds—Department of Plant Sciences. Applications are invited for the post of Grade 4 Technician in Photography. The person appointed will be in charge of Departmental Photography, including enlargement printing for light and electron microscopy, reduction printing for lantern slides, colour and B/W photography of plant material, and general care and supervision of all darkroom facilities. Applicants should possess an appropriate qualification and have had about seven years relevant experience including a training period. Salary on the scale £2,955 to £3,402 a year. Applications with the names of two referees and full details of qualifications and practical experience should be submitted to The Secretary, Department of Plant Sciences, Baines Wing, The University, Leeds LS2 9JT.

8133(A)

ECOLOGY

YALE UNIVERSITY

DEPARTMENT OF BIOLOGY

seeks candidates for senior and junior appointments in Ecology. The appointees will be expected to contribute to a strong program of research and scholarship in ecology, and participate in undergraduate and graduate teaching.

Send curriculum vitae, résumé of past and present research, and names of referees to: The Chairman, Department of Biology, 102 KBT, Yale University, New Haven, Connecticut 06520. Deadline for receipt of applications is June, 1979.

Yale University is an Equal Opportunity Affirmative Action Employer. We invite applicants from all qualified individuals, especially women and members of minority groups.

8164(A)

POSTDOCTORAL
RESEARCH POSITION
IN THE DEPARTMENT OF
BIOCHEMISTRY, MEDICAL
COLLEGE OF VIRGINIA
VIRGINIA
COMMONWEALTH
UNIVERSITY

Richmond, Virginia, U.S.A.

Applications are invited for a post-doctoral position available from February 1, 1979, for research on the turnover of specific enzymes and characterisation of proteases in tissues of normal or diabetic animals. Salary \$10,000 per annum. Applications, including a curriculum vitae and the names and addresses of two referees, should be sent prior to December 1, 1978 to Dr J. S. Bond, Strangeways Research Laboratory, Wort's Causeway, Cambridge CB1 4RN.

8150(A)

CHANGE OF ADDRESS

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3 DYERS BUILDINGS, LONDON, EC1N 2NR
TELEPHONE : 01 831-6901

AGRICULTURAL RESEARCH COUNCIL ANIMAL BREEDING RESEARCH ORGANISATION

Applications are invited for a vacancy in the Department of Growth and Efficiency for work on the genetics of growth and productive efficiency of livestock.

Candidates should have a first or upper second class Honours Degree or the equivalent in an appropriate subject and at least two years' post-qualifying experience in mathematics, statistics, genetics or animal science. They should have the ability to conduct and direct large-scale experiments on cattle fed a controlled complete diet. An ability to analyse data and use extensive computing facilities is essential.

Appointment as Higher Scientific Officer (£4,101 to £5,448), starting salary depending on experience. Non-contributory superannuation.

Further information and application forms are available from the Secretary, ARC Animal Breeding Research Organisation, West Mains Road, Edinburgh EH9 3JQ. Closing date November 11, 1978. 8012(A)

UNIVERSITY OF CAMBRIDGE DEPARTMENT OF ZOOLOGY

Applications are invited for two Demonstratorships for persons working in the fields of Behavioural Ecology and Vertebrate Physiology respectively, to take up appointment by October 1, 1979. Appointments will be for three years, with the possibility of re-appointment for two years. Proposed pensionable stipend £4,133 to £5,129 according to age and experience.

Further information about Cambridge Demonstratorships and about the duties and conditions of appointment may be obtained from the Secretary of the Appointments Committee, Faculty of Biology "A", Department of Applied Biology, Pembroke Street, Cambridge CB2 3DX, to whom candidates should send their applications (six copies), together with curriculum vitae and the names of two or three referees, by January 12, 1979. 8156(A)

UNIVERSITY OF KENT AT CANTERBURY BIOLOGICAL LABORATORY

Protozoology Group
Applications are invited for the following research posts.

POSTDOCTORAL RESEARCH FELLOW

to work on the biochemistry of pyrimidine biosynthesis in *Trypanosoma cruzi*. Preference will be given to candidates with experience in enzymology or nucleic acid metabolism. Salary scale £3,883 to £6,555.

GRADUATE RESEARCH ASSISTANT

to work on various aspects of the biochemistry of *Trypanosoma cruzi*. Candidates with degrees in biochemistry will be preferred. Salary scale £3,384 to £4,882.

The appointments will be initially for one year with a good prospect of extension for a further period of two years. Training in protozoological techniques will be provided.

Application forms and particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, The University, Canterbury, Kent CT2 7NH, to whom completed applications should be returned by November 17, 1978. Please quote ref. A37/78. 8105(A)

TECHNICIAN GRADE 3, required in M.R.C. Neuroimmunology Project. Work will involve the identification, purification and *in vitro* culture of cells from nervous tissue using immunological and biochemical techniques. Salary in range £3,153 to £3,525 inc. of London Weighting (under review). This appointment is supported by grant funds. Application form from Personnel Officer (Technical Staff CM17), University College London, Gower Street, London WC1E 6BT. 8152(A)

TECHNICIAN Grade 5, with knowledge of Cytology required to assist in both the research and undergraduate teaching laboratories in the Dept. of Botany and Microbiology. It is essential for applicants to have a sound knowledge of Cytological Techniques. Salary according to experience in range £3,651 to £4,185 inc. of London Weighting (under review). Application form and further details from Personnel Officer (Technical Staff CD9), University College London, Gower Street, London WC1E 6BT. 8153(A)

Please mention
Nature
when replying to
these advertisements

Applications are invited for the position of:

PROJECT LEADER

in a DRUG METABOLISM AND PHARMACOKINETICS GROUP with a leading pharmaceutical company in Paris.

The applicant should have a particular knowledge of h.p.l.c. methods of analysis and a PhD degree in a relevant discipline.

Send applications and CV to NATURE, Box No 8106(A), 3, Dyers Buildings, London EC1N 2NR.

All replies will be treated in the strictest confidence. 8106(A)

ROTHAMSTED EXPERIMENTAL STATION Harpenden, Herts. AL5 2JQ BIOCHEMIST

In Soil Microbiology Department to study the physiology of VA endophytes and the possibility of their culture in artificial media. The post is tenable for approximately 3 years and is funded by the Ministry of Overseas Development.

Qualifications: First or upper second class Honours degree or the equivalent and at least two years' postgraduate experience. Preference will be given to candidates with appropriate post-doctoral experience in plant or microbial biochemistry and/or root physiology.

Appointment in grade of Higher Scientific Officer (£4,101 to £5,448) or Senior Scientific Officer (£5,154 to £6,898), grade and starting salary depending on experience. At least four years' postqualifying experience would normally be required for appointment as S.S.O. Non-contributory superannuation.

Apply in writing to the Secretary, giving names and addresses of two referees and quoting Ref 374 by November 24, 1978. Further details on request. 8172(A)

PRE AND POSTDOCTORAL POSITIONS

are available for studies on the protein synthesizing system with special emphasis on ribosomes—topography, biogenesis and function—under various conditions of growth and development. Teaching opportunities available. Reply with résumé to:

Professor Bruce H. Sells,
Laboratories of Molecular Biology,
Faculty of Medicine,
Health Sciences Complex,
Memorial University
of Newfoundland. 8003(A)

THE MEDICAL COLLEGE OF ST BARTHOLOMEW'S HOSPITAL

DEPARTMENT OF OBSTETRICS AND GYNAECOLOGY

Applications are invited for a Research Assistant to participate in a research programme on Cancer of the Ovary. This post in the Williamson Laboratory is suitable for a Science Graduate, preferably with some experience in laboratory immunology. The post is funded by a grant from the Cancer Research Campaign which is subject to renewal.

Further particulars may be obtained from: Mr T. A. Poulton, Williamson Laboratory and applications should be sent to The Secretary, The Medical College of St Bartholomew's Hospital, West Smithfield, London EC1A 7BE, quoting reference 807. 8098(A)

INTERNATIONAL INSTITUTE OF HUMAN NUTRITION TECHNICIANS

for lipid analyses and for clinical biochemistry.

Applications stating qualifications, age and experience to Director, International Institute of Human Nutrition, High Street, Sutton Courtenay, Abingdon, Oxon OX14 4AW. 8165(A)

UNIVERSITY OF RHODES DEPARTMENT OF GEOGRAPHY SENIOR LECTURESHIP ON LECTURESHIP IN GEOGRAPHY

Applications are invited for one post in human geography tenable from January 1979. Preference may be given to persons whose special fields of study include one or more of the following: cultural, urban, political geography. Interested persons should indicate their applications the full range of their interests and abilities in teaching and research, including any pertinent to regional courses.

Salary Scales (approx. std. equiv. Senior Lecturer, £7,544 to £9,900; Lecturer Grade I, £6,843 to £7,877; Lecturer Grade II, £4,224 to £6,611). Both permanent, pensionable terms of short-term one- or two-year contracts are offered.

Further particulars and application procedure should be obtained from the Association of Commonwealth Universities (AppUs), 36 Gordon Square, London WC1H 0PF.

Closing date for receipt of applications: November 30, 1978.

British subjects considering applying for posts in Rhodesia are urged to consult the Foreign and Commonwealth Office (telephone 233 4143), their nearest British Consular Office. 8081(A)

MEDICAL RESEARCH COUNCIL NATIONAL INSTITUTE FOR MEDICAL RESEARCH DIVISION OF VIROLOGY

A scientific staff vacancy exists in a small team studying the mechanism of virus infection using the adenovirus model system. We require someone who has had experience in virologic and/or biochemical techniques as well as some expertise in gene engineering (our Category III laboratory will be available shortly).

The appointment will be for up to three years and will be in the salary range £4,655 to £6,090 per annum according to age and qualification plus £450 per annum London Allowance.

Applications, giving details of qualifications, experience and the names of two professional referees, should be sent to the Director, National Institute for Medical Research, Mill Hill, London NW7 1AA before November 30, quoting reference V.1. 8096(A)

WEST OF SCOTLAND HEALTH BOARDS DEPARTMENT OF CLINICAL PHYSICS AND BIO-ENGINEERING POSTDOCTORAL RESEARCH WORKER

Applications are invited for a funded post to study the uptake, methylation and excretion of mercury in biological systems, with special reference to mercury hazards in food practice. Experience of G.L.C. would be advantageous.

Salary scale £3,915 to £4,395 p.a. annum (three year appointment N.H.S. superannuation scheme).

Application form and job description may be obtained from the Director, Department of Clinical Physics and Bio-Engineering, 11 West Graham Street, Glasgow G4 9LF, to whom completed applications should be returned by November 30, 1978. 8100(A)

CHANGE OF ADDRESS

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TELEPHONE: 01 831-8901

**UNIVERSITY OF
HONG KONG
DEPARTMENT OF
ANATOMY
SENIOR LECTURESHIP
IN ANATOMY**

Following the establishment of a tal School, applications are invited appointment from September 1, to a post of Senior Lecturer in tomy. Applicants should be either dental, ical or science graduates with rience in teaching anatomy to linical dental students. The intment carries the responsibility coordinating and implementing all omical teaching within the dental rse. The appointee will be expected ngage in research work and may be ired to teach other students on ision. Facilities exist within the artment for a major research rest to be built up in one of the biology fields. Annual salary (superannuable) is:— \$98,700 by 4,260 to 120,000 by 0 to 132,600. (£1 equals HK\$9.30 rox). Starting salary will depend qualifications and experience. Further particulars and application ns may be obtained from the ocation of Commonwealth (Appts), Gordon Square, London WC1H , or the Assistant Secretary cruitment), University of Hong ig, Hong Kong. Closing date for applications is ember 30, 1978. 8140(A)

**ST GEORGE'S HOSPITAL
MEDICAL SCHOOL
(University of London)
RESEARCH ASSISTANT
IN
BIOCHEMISTRY**

ired to work in the field of ation, characterisation and mical modification of biologically ve peptides and macromolecules ted to human pancreatic and ignant disorders. Some previous rience in chemical modification of eins desirable but not essential. A successful candidate will be ected to register for a Ph.D. ee. The appointment, which is in Surgery Department, will be for e years. Emoluments according to erience; minimum starting £3,834 annum. Further details and lication forms available from the blishment Officer, St George's pital Medical School, Cranmer ace, London SW17 0RE. Closing November 19. 8141(A)

**UNIVERSITY OF READING
DEPARTMENT OF PHYSIOLOGY
AND BIOCHEMISTRY
RESEARCH
DEMONSTRATORSHIPS**

Applications are invited from gradu- in Biochemistry, Physiology, or r related subjects, who would nor- y have an upper second class purs degree, for a Research Dem- ratorship to carry out research for igher degree in one of the following ects: regulation of hepatic metabolism; lies in isolated cells and the used organ; he effect of DNA strain-breaks on activity of the chromosomal yme poly(ADP-ribose) polymerase cultured mammalian cells. he support is for three years with possibility of an extension and the es include up to ten hours demon- ting per week during term time. ry in scale £3,384; £3,634; £3,883 annum. nquiries and/or applications, in- ling the names of two academic ees, should be addressed to Pro- r G. M. H. Waites, Department Physiology and Biochemistry, Uni- ty of Reading, Whiteknights, ding RG6 2AJ. Telephone Reading 23, extension 7675. (Ref. TN70 A.) 8146(A)

**UNIVERSITY OF OXFORD
DEPARTMENT OF GEOLOGY
AND MINERALOGY
POSTDOCTORAL
RESEARCH ASSISTANT
IN PALAEOLOGY**

A Postdoctoral Research Assistant is required for a period of up to two years to work under the direction of Dr W. J. Kennedy on an N.E.R.C. supported project on the evolution and biogeography of European late Creta- ceous ammonite faunas. Starting salary will be in the range £3,383 to £4,882 per annum depending on age and experience, plus U.S.S. benefits. Applications, together with the names of two referees should be sub- mitted as soon as possible, and not later than November 14, 1978 to Dr W. J. Kennedy, Department of Geology and Mineralogy, Parks Road, Oxford, from whom further particulars may be obtained. 8162(A)

**UNIVERSITY OF GLASGOW
CHAIR OF GENETICS**

Applications are invited for the Chair of Genetics which will become vacant on March 1, 1979. Further particulars may be had from the Secretary of the University Court (Room 18), The University of Glasgow, Glasgow G12 8QQ, with whom applications (15 copies) giving the names and addresses of three referees, should be lodged on or before December 15, 1978. In reply please quote Ref. No. 4268M. 8174(A)

**UNIVERSITY OF GLASGOW
LECTURESHIP IN
ANIMAL HUSBANDRY**

IN THE VETERINARY SCHOOL. Applications are invited for the appointment of a Lectureship in the Department of Animal Husbandry. The salary scale will be within the range £3,883 to £7,754 per annum (under review) of the Lecturers' scale. Initial placement will be according to qualifications and experience. Candidates should be either Veter- inary graduates or agricultural science graduates with a developed interest in farm management and/or production. Further particulars may be had from the Secretary of the University Court (Room 18), The University of Glasgow, Glasgow G12 8QQ, with whom applications (eight copies) giving the names and addresses of three referees, should be lodged on or before November 17, 1978. In reply please quote Ref. No. 4266M. 8148(A)

**UNIVERSITY OF ASTON
IN BIRMINGHAM**

DEPARTMENT OF CHEMISTRY. Applications are invited from suit- ably qualified candidates for two lectureships in the Polymer Section: (1) A Lecturer in polymer technology is required to teach and conduct research in the field of polymeric materials. Preference will be given to candidates with knowledge of the design and selection of polym- ers for commercial and ind- ustrial uses. (2) A Lecturer in polymer engineer- ing/physics is required to teach and conduct research in the field of materials engineering/science. Commencing salary will be within the range £3,883 to £5,842 per annum on a scale rising by annual increments to £7,754 per annum (under review). Application forms and further par- ticulars may be obtained from The Staff Officer (Ref. 311/6), University of Aston in Birmingham, Gosta Green, Birmingham B4 7ET (Tel. 021 359 3611, Ext. 201), to whom applications should be forwarded not later than November 20, 1978. 8149(A)



Open to both
men and women

Public Service
Canada
Fonction publique
Canada

PALEONTOLOGIST

Salary: \$16,500 to \$39,517 (depending on experience)
Ref. No: 78-RES-63 (N)

**Energy, Mines and Resources, Geological Survey of Canada
Calgary, Alberta**

A scientist is required at the Institute of Sedimentary and Petroleum Geology to conduct research in Western and Northern Canada on marine molluscan faunas from the Cretaceous period. The successful candidate will investigate phylogenetic relationships of fossils through detailed field and taxonomic studies, and will develop and apply biostratigraphic models for paleoecological assessment and for local, regional and world-wide correlation.

Qualifications

Applicants must possess a Ph.D., or have a lesser degree with evidence of research experience and productivity equivalent to a doctorate in geology or paleontology with specialization in molluscan faunas. Experience and demonstrated ability in paleontological studies of Cretaceous cephalopods and pelecypods is essential. Knowledge of English is essential.

**RESEARCH SCIENTIST —
ORGANIC GEOCHEMISTRY**

Salary: \$15,230 to \$36,760 (depending on experience)
Ref. No: 77-PSTP-32-153 (N)

**Fisheries and Environment Canada
Burlington, Ontario**

A research scientist is required in the Process Research Division of the National Water Research Institute to study the physical-chemical processes involving organic matter in recent and modern sediments associated with aqueous systems. Emphasis will be placed on the carbon cycle and studies will be undertaken in lakes and rivers in support of national water management programs. The incumbent also provides advice and assistance in the development of optimal water resource management strategies.

Qualifications

Applicants must possess a Ph.D. or lesser degree with evidence of research experience and productivity equivalent to that of the Ph.D. in geochemistry or a closely related field. Experience and research productivity in the field of organic geochemistry is also required. Knowledge of English is essential.

How to Apply

Send your application form and/or résumé to:
**Mr. D.E. Fraser
National Capital Region Staffing Office
Public Service Commission of Canada
Ottawa, Ontario K1A 0M7
Closing Date: November 17, 1978**

8077(A)

Please quote the applicable reference number at all times.

THE DEPARTMENT OF ZOOLOGY
of the University of California, Davis
announces the vacancy of an
**ASSISTANT
PROFESSORSHIP**
in the area of
**DEVELOPMENTAL
BIOLOGY**

The Department of Zoology invites applications from persons with primary interests and experience in research and teaching in any area of developmental biology. The appointee is expected to establish a productive research program and to participate in the department's program of graduate instruction. Teaching responsibilities include a one-quarter introductory course in embryology, a one-quarter advanced course in developmental biology, and participation in a graduate seminar in developmental biology. Candidates must have a Ph.D. degree. Salary will depend upon qualifications and experience.

Applicants should forward a curriculum vitae, a description of their research experience and future research plans, reprints of all publications, a summary of teaching capabilities, and three letters of recommendation to Dr. R. D. Grey, Chairperson of the Search Committee, Department of Zoology, University of California, Davis, California 95616. The University of California is an equal opportunity/affirmative action employer. Closing date for receipt of applications is November 30, 1978.

COMPLIANCE STATEMENT

The University of California, Davis, in compliance with Titles VI and VII of the Civil Rights Act of 1964, Title IX of the Education Amendments of 1972 (45 CFR 86), and Sections 503 and 504 of the Rehabilitation Act of 1973, does not discriminate on the basis of race, color, national origin, religion, sex, or handicap in any of its policies, procedures, or practices, nor does the University, in compliance with the Age Discrimination in Employment Act of 1967 and Section 402 of the Vietnam Era Veterans Readjustment Act of 1974, discriminate against any employee or applicants for employment on the basis of their age or because they are disabled veterans or veterans of the Vietnam era. This nondiscrimination policy covers admission, access, and treatment in University programs and activities, and application for and treatment in University employment. In conformance with University policy, and pursuant to Executive Orders 11246 and 11376, Section 503 of the Rehabilitation Act of 1973, and Section 402 of the Vietnam Era Veterans Readjustment Act of 1974, the University of California is an affirmative action/equal opportunity employer. Inquiries regarding the University's equal opportunity policies may be directed to the Vice Chancellor of Academic Affairs and Affirmative Action Officer, 515 Mraz Hall, 752-2070, the Vice Chancellor of Student Affairs and Title IX Compliance Officer, 541 Mraz Hall, 752-2417, the Rehabilitation Act Compliance Coordinator, Office of the Chancellor, 581 Mraz Hall, 752-6560, or the Director, Office of Civil Rights, DHEW 8189(A).

**UNIVERSITY OF
NOTTINGHAM
DEPARTMENT OF
CHEMISTRY
MASS SPECTROMETER
TECHNICIAN GRADE 5**

Applications are invited for this post which involves the operation and maintenance of an MS902 mass spectrometer. This post offers interest in an important aspect of the analytical services required by the Department. Experience in the use of mass spectrometers or in the general field of analytical instrumentation would be desirable although not essential. Excellent working conditions are offered with a starting salary within the scale £3 186 to £3 720 p.a. (under review).

Applications in writing giving full details and the names of two referees should be sent to the Establishment Officer, University of Nottingham, University Park, Nottingham NG7 2RD 8081(A).

**RESEARCH FELLOW
IN PSYCHOLOGY
RESEARCH PSYCHOLOGIST**

required for one year from December 1, 1978 for a study of the behavioural aspects of infant feeding based in the JOHN RADCLIFFE HOSPITAL, OXFORD. The project is supported by the International Council for the Infant Feeding Industries and supervised by Dr R. F. Drewett (Department of Psychology, University of Durham) and Dr J. D. Baum (Department of Paediatrics, University of Oxford). The appointee will be a Research Fellow of the University of Durham, working in Oxford. Applicants should have a Ph.D. or equivalent research experience in psychology or a related subject.

Salary in the range £3,883 to £4,382 per annum on National Range 1A plus superannuation.

Applications (3 copies) naming three referees should be sent by November 10, 1978, to the Registrar and Secretary, Science Laboratories, South Road, Durham DH1 3LE, from whom further particulars may be obtained 8083(A).

FELLOWSHIPS

**UNIVERSITY OF
BIRMINGHAM
DEPARTMENT OF
ZOOLOGY AND COMPARATIVE
PHYSIOLOGY
S.R.C. RESEARCH
FELLOWSHIP**

Applications are invited for a Postdoctoral Research Fellowship to work on the function of avian retinal oil droplets. The research will involve the determination of oil droplet characteristics and visual capacities in birds, with a view to exploring the relationships between sensory physiology, visual capacities and behavioural ecology. Previous experience with animal training procedures would be an advantage.

Candidates would have a Ph.D. in either Zoology, Psychology or Physiology Appointment, for three years from December 1, or as soon as possible thereafter, on the Research Fellow Grade 1A, £3,883 to £6,555, plus superannuation. Maximum starting salary £4,382.

Further particulars from the Assistant Registrar (Science and Engineering), University of Birmingham, P.O. Box 363, Birmingham B15 2TT, to whom applications (three copies) containing full curriculum vitae and naming three referees should be sent by Friday, November 10, 1978. Please quote ref. NJ6 8125(F).

**POSTDOCTORAL
FELLOWSHIPS**

Two three-year postdoctoral fellowships are available at the Fried Miescher-Institut, Basel, in a study correlating between structure and function in synaptic junctions from mammalian brain.

Biochemist. Preferably experienced in membrane biology, sub-cell fractionation and gel electrophoresis. Experience in immunochemistry, protein chemistry would be relevant. A grasp of the principles of light and electron microscopy would have to be developed and appropriate training will be given where necessary.

Electron Microscopist. Prefers with experience of CNS ultrastructure. Experience of quantitative methods immunohistochemical procedures would be relevant. Candidates should be prepared to utilize light microscopy; classically should a particular problem demand it.

Reply to Dr A. I. Matus, Friedrich Miescher-Institut, P.O. 1 273, CH-4002 Basel, Switzerland sending a brief cv and names of three referees 8163(E).

**UNIVERSITY OF
STRATHCLYDE**

Applications are invited for a
**POSTDOCTORAL
RESEARCH FELLOWSHIP**
supported by S.R.C. in the
**DEPARTMENT OF PURE AND
APPLIED CHEMISTRY**

from mathematicians, physicists or chemists to carry out theoretical studies of dynamical properties of polymer.

Appointment on Range 1A of the national salary structure for research and analogous staff, commencing salary within the range £3,883 to £4,382 with placing according to qualifications and experience. Superannuation benefits.

Duration of appointment will be for two years.

Written applications naming two referees (quoting R42/78) should be sent direct to Dr D. Pugh, Department of Pure and Applied Chemistry, University of Strathclyde, Thomas Graham Building, 295 Cathedral Street, Glasgow G1 1XL, from whom further information can be obtained (tel 041 552 4400, ext 2290) 8167(E).

**UNIVERSITY OF SUSSEX
School of Biological Sciences
POST DOCTORAL
FELLOWSHIP IN
NEUROBIOLOGY**

Applications are invited for a 3 year S.R.C. Postdoctoral Research Fellowship to investigate the neural basis for stimulus localisations by the lateral line system in XENOPUS. Previous neurophysiological experience in recording from the central and peripheral nervous system is required and some knowledge of techniques in the field of developmental neurobiology is desirable.

Salary on the Research Fellow Grade 1A Scale £3,883 to £6,555 p.a. (under review) with superannuation benefits.

Applications, giving details of age, qualifications and experience, together with the names of 2 referees, should be sent as soon as possible to Dr I. J. Russell, School of Biological Sciences, University of Sussex, Falmer, Brighton, Sussex, from whom further particulars are available (telephone Brighton 606755 ext 480) 8120(E).

**UNIVERSITY OF
SOUTHAMPTON
CRYOGENICS RESEARCH
POSTDOCTORAL FELLOWSHIP**

Applications are invited for the following post commencing as soon as possible for one year in the first instance.

**HELIUM REFRIGERATION
IN THE ROTATING FRAME**

To study heat transfer and fluid behaviour of helium at 3000 r.p.m. and develop stable refrigeration cycles for larger superconducting machines. A 1 meter rotating cryostat is available for experimental studies.

Applicants should have experience in mechanical/chemical engineering or low temperature physics.

Salary in range of £3,883 to £5,367 per annum.

Applications, giving details of age, experience, etc., and naming two referees should be sent to D. A. S. Copland, The University, Southampton SO9 5NH quoting reference 383/R/Na. 8128(E).

**UNIVERSITY OF GLASGOW
DEPARTMENT OF GENETICS
M.R.C. POSTDOCTORAL
FELLOWSHIP**

Applications are invited for a M.R.C.-funded Postdoctoral Research Fellowship to study the antigenic composition of the human trophoblast (placental) plasma membrane and relevance to immunological relations between mother and fetus. The facilities and expertise of the Institute of Genetics in cell hybridisation will be used to make monoclonal antibodies against placental antigens. Applicants should have experience in biochemistry, immunology, or immunohistochemistry.

Starting salary according to age and qualifications up to £4,382 p.a. on range 1A of the salary scale Research and Analogous Staff.

Enquiries and applications, including two copies of curriculum vitae, names and addresses of two referees to Dr R. G. Sutcliffe, Department of Genetics, University of Glasgow G 5JS, U.K.

In reply please quote Reference No. 4267/M. 8147(E).

FELLOWSHIPS

UNIVERSITY OF BIRMINGHAM DEPARTMENT OF PLANT BIOLOGY POSTDOCTORAL FELLOWSHIP

Applications are invited for a Post-doctoral Research Fellowship supported by the Ministry of Overseas Development which is tenable for one year from January 1, 1979 to work on storage of cocoa seeds. Salary in the range £3,883 to £6,555 plus superannuation. Starting salary will not exceed £4,382.

Further particulars are available from the Assistant Registrar, Science and Engineering, P.O. Box 363, Birmingham B15 2TT to whom applications (see copies) including a curriculum vitae and the names and addresses of three referees should be sent by January 10, 1978.

Please quote ref: NK5.

8126(E)

THE ROYAL SOCIETY BRUNO MENDEL FELLOWSHIPS

Applications are invited for Bruno Mendel Fellowships for one year from October 1, 1979, renewable for one year exceptionally two additional years, for postgraduate candidates normally resident in the United Kingdom, The Netherlands, or Israel, to carry out research in medicine or the biomedical sciences at a university, medical research centre or medical school. Ends will normally be at the level of junior research fellowship appointment in the United Kingdom; additional allowances for travel and subsistence may be provided as appropriate. Application forms, received by January 15, 1979, and nominations (revised in May 1976) are available from the Executive Secretary, Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.

8135(E)

UNIVERSITY OF KENT AT CANTERBURY LECTURESHIP AND TUTORIAL FELLOWSHIP IN MICROBIOLOGY

Applications are invited for appointment to a vacant Lectureship and a newly created Tutorial Fellowship. Preference will be given to candidates with research interests in either microbial physiology or microbial ecology. It is expected that the Lecturer will be appointed within the first five points on the salary scale £3,883 to £7,764, and the Tutorial Fellow within the first three points of scale £3,883 to £6,555. The latter is a fixed term appointment normally of three years duration. Application forms and particulars may be obtained from the Senior Assistant Registrar, Faculty of Natural Sciences, Chemical Laboratory, Canterbury, Kent to whom completed applications should be returned by November 24, 1978. Please quote ref: A38/78.

8118(E)

UNIVERSITY OF SOUTHAMPTON DEPARTMENT OF PHYSICS CRYOGENICS Research Fellowship

Applications are invited for a three year postdoctoral fellowship to study heat transfer in the reboiler/condensers of cryogenic plant, using model rigs including a full-length test section. Starting salary up to £5,129 per annum plus U.S.S. benefits. Applicants with experience in fluid heat transfer, not necessarily at low temperatures, should send a curriculum vitae with the names of two referees to D. A. S. Copland, The University, Southampton SO9 5NH, quoting reference 156/R./Na.

8131(E)

Science Research Council ANGLO-AUSTRALIAN OBSERVATORY TWO POST-DOCTORAL RESEARCH FELLOWSHIPS

The Anglo-Australian observatory (AAO) comprises the 3.9 metre telescope near Coonabarabran, N.S.W. and a laboratory at Epping near Sydney, Australia.

The U.K. Science Research Council provides two post-doctoral research fellowships tenable at the AAO. Candidates should have strong backgrounds in some branch of physics or astrophysics, including theory. The appointment will normally be for two years to start in October 1979. Extension of the fellowships for a third year will be available if the fellow wishes to spend an additional year at an institution in the U.K. Salary will be based on the U.K. university lecturer scale in the range £3,883 to £7,754 (subject to review in 1979) plus other allowances, fares, etc.

Applications should include:

1. Age, citizenship, country of residence and marital status.
2. Qualifications and experience, including date of Ph.D.
3. Astronomical research projects for which telescope time will be sought. (Sufficient information is required to show that the proposal would be competitive in applications to the panel for the allocation of telescope time).
4. Any instrument development project in which the applicant is particularly interested.
5. When applicant would be available.
6. Names and addresses of three referees.

Applications (Quoting reference RF/NS) should be sent to Miss F. Brandon, Science Research Council, Advance Office, P.O. Box 18, Swindon SN2 1ET, England. Telephone: Swindon 26222, Ext. 2226. Applicants should arrange for the three references to be sent to the above address by the closing date of 5th January, 1979.

8143(E)

STUDENTSHIPS

THE ROYAL SOCIETY JOHN MURRAY TRAVELLING STUDENTSHIP IN OCEANOGRAPHY AND LIMNOLOGY 1979

Applications are invited for the Murray Studentship, open to British citizens under the age of 35, for the purpose of travel and work in oceanography or limnology. This award, which may be held for periods of three months to one year, will in 1978 be up to £1,500, adjusted according to qualifications and place of residence.

Applications are especially invited from workers in oceanography and limnology, as well as in zoology, botany, geology, mathematics and physics, and it will be permitted to the award while receiving a salary from a regular appointment.

Applications receivable by December 1978, should be made on forms available from The Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AG.

8134(F)

UNIVERSITY OF NEWCASTLE UPON TYNE DEPARTMENT OF AGRICULTURAL BIOCHEMISTRY S.R.C. C.A.S.E. STUDENTSHIP

Applications are invited for a C.A.S.E. Studentship in the following topic:

Continuous Fermentation Studies with Rumen Microorganisms

Applicants should have at least an Upper Second Class Honours Degree in either Bacteriology, Microbiology, Applied Biochemistry or a related subject. The project, which is sponsored by I.C.I. Pharmaceuticals will be supervised by Professor D. G. Armstrong and Mr D. G. Harrison, from whom full details are available.

Applications, containing the names of two referees, should be sent to Professor D. G. Armstrong, Department of Agricultural Biochemistry, University of Newcastle upon Tyne, Newcastle upon Tyne NE1 7RU by November 13, 1978.

The studentship must be taken up by March 31, 1979.

8080(A)

MICROBIOLOGY graduate required for a Ph.D. Studentship in connection with a research project on bacteriological aspects of acne. Apply in writing, with full curriculum vitae and the names of two referees, to the Secretary, Guy's Hospital Medical School, London Bridge, SE1 9RT, quoting Ref. M.C.6.

8094(A)

THE UNIVERSITY OF MANCHESTER DEPARTMENT OF BIOCHEMISTRY S.R.C. C.A.S.E. STUDENTSHIP

Applications are invited from good Honours graduates with enterprise, initiative and interests at the interface of Biochemistry and Organic Chemistry to work for three years on the covalent attachment of fluorescent labels to carbohydrates. This is a joint project with the Organics Division of I.C.I. at Blackley, Manchester. The appointment will commence as soon as possible and preferably not later than December 31, 1978. Apply as soon as possible, with curriculum vitae, telephone number, and names and addresses of two referees, to Dr J. S. Thompson, Department of Biochemistry, University of Manchester M13 9PL (Telephone (061) 273 7121, ext. 5232 or 5108).

8085(A)

UNIVERSITY OF SOUTHAMPTON C.A.S.E. Studentship in CRYOGENICS

Applications are invited for a 2 year S.R.C. C.A.S.E. Research Studentship on "Refrigeration cycles for rotating helium at 3000 r.p.m."

Applicants with a good degree in Physics or Engineering should write to Dr R. G. Seurlock, Physics Department, University of Southampton, as soon as possible. Please quote ref: MA1/2/Na.

8127(A)

UNIVERSITY OF MANCHESTER DEPARTMENT OF BACTERIOLOGY AND VIROLOGY

The North West Water Authority is funding a three-year postgraduate studentship in microbiology which will be primarily concerned with the development of standard methods for the quantitative estimation of salmonellae strains in sewage sludge. Maintenance grant payable: £1,860 per annum.

The successful candidate will register for a higher degree, probably at M.Sc. level for one year but renewable for a further two years for a Ph.D. subject to satisfactory academic progress. The award is tenable as soon as possible. Particulars from the Registrar, The University, Manchester M13 9PL (quote ref. 244/78/N) to whom letters of application should be sent by November 14.

8160(F)

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AWARDS

Awards for RESEARCH INTO TROPICAL RESPIRATORY DISEASES

The Wellcome Trust wishes to encourage research into diseases of the respiratory system, including asthma, occurring in the tropics. A competitive award of up to £100,000 is offered for a period of not more than five years. Preference will be given to proposals which demonstrate an interdisciplinary approach and especially those which include sustained collaboration with research centres in the tropics.

Applications for smaller awards will also be considered and grants may also be made to the runners up. *These awards are additional to the Trustees' normal arrangements for supporting tropical medical research.*

Those wishing to apply should send a two-page summary of their objectives and proposals for research, including a brief estimate of the anticipated costs, for preliminary consideration. The principal scientist should also send a curriculum vitae.

Applications should be addressed to **Mr. M. A. F. Barren, The Wellcome Trust, 1 Park Square West, London NW1 4LJ, and reach the Trust by 8th December 1978.**

The Wellcome Trust

8177(N)

CONFERENCES

CONFERENCE

Third International Conference on the Chemistry and Uses of Molybdenum

sponsored by the University of Michigan and Climax Molybdenum Co. Ann Arbor, Michigan, U.S.A., August 19-23, 1979.

Application forms can be obtained from:

Climax Molybdenum Co. Ltd.,
Imperial House,
1/3 Grosvenor Place,
London SW1X 7DL. 8034(C)

FOR SALE & WANTED

J. Physiol. Vols 107 (1948) to 198 (1968) and 236 (1974) to 282 (1978). Offers to Box No. 8144(L) Macmillan Journals Ltd., 3 Dyers Buildings, Holborn EC1N 2NR. 8144(L)

ATMOSPHERIC PRESSURE RECORDS

If you have any such records in any form for any part of the world, for any part or all of any of the periods listed below, please send a copy to Dr B. W. Jones, Faculty of Science, The Open University, Milton Keynes MK7 6AA, Gt. Britain. Reasonable expenses can be recovered. *Periods:* March 1 to 14, 1970; July 4 to 17, 1972; June 24 to July 7, 1973; June 14 to 27, 1974; October 17 to 30, 1976; October 6 to 19, 1977. 8157(L)

SYMPOSIA

THE SECOND INTERNATIONAL SYMPOSIUM ON MICROBIAL ECOLOGY

will be held
at the University of Warwick,
Coventry, England
September 7-12, 1980

For information and a copy of the First Circular, contact: Dr M. Fletcher, Department of Environmental Sciences, University of Warwick, Coventry CV4 7AL, England. 8078(M)

SYMPOSIA

INTERNATIONAL PEPTIDE SYMPOSIUM

The Intramural Program of the National Institute of Neurological and Communicative Disorders and Stroke will sponsor an International Symposium entitled "The Role of Peptides in Neuronal Function" to be held May 7-9, 1979, at the National Institutes of Health reservation in Bethesda, Maryland. The three-day meeting will draw together investigators from diverse disciplines for a comprehensive review of peptide research as it relates to the field of neurobiology.

The first day will include presentations on the anatomy and physiology of peptidergic neurons, as well as the synthesis and secretion of peptides. The second day will begin with a discussion of peptide-mediated communication in the nervous system, followed by reviews of the neurobiology of specific peptides, including angiotensin, neurotensin, somatostatin, substance P, vasoactive intestinal peptide, luteinizing hormone releasing hormone, thyrotropin releasing hormone, olfactory system peptide, bag cell hormone and neurodepressing hormone. The final day will be devoted entirely to the pharmacology, physiology and potential clinical application of opioid peptides.

The Symposium is being organised by Drs Jeffery Barker and Thomas Smith of the N.I.N.C.D.S. Laboratory in Neurophysiology.

Those interested in attending should contact either Dr Barker or Dr Smith, Bldg. 36, Room 2C02, National Institutes of Health, 9000 Rockville Pike, Bethesda, Md. 20014. Tel: 301-496-2414. 8168(M)

GRANTS

THE ROYAL SOCIETY BROWNE RESEARCH FUND MAURICE HILL RESEARCH FUND MARSHALL AND ORR BEQUEST Grants 1979

The Council of the Royal Society invites applications from qualified research workers for personal grants:

(a) from the Browne Research Fund, for research in marine biology at a marine biological laboratory or elsewhere. Preference will be given to those who propose to work on purely scientific problems rather than to those whose work would be primarily directed towards an economic end.

(b) from the Maurice Hill Research Fund (established in memory of the late Dr M. N. Hill, F.R.S., for the encouragement of research in physical oceanography and marine geophysics) for research at sea and/or in the laboratory in physical and chemical oceanography, marine geophysics and geology.

(c) from the Marshall and Orr Bequest for the support of marine biological research.

Grants may be used for periods up to one year, and are made on the understanding that university or other posts held by applicants will meanwhile be kept open for them. Recipients are permitted to use grants while receiving salaries from regular appointments.

In 1979 the total amounts available to be shared among all successful applicants will be about £4,250 from the Browne Research Fund, £8,400 from the Maurice Hill Research Fund and £7,820 from the Marshall and Orr Bequest; individual grants will not ordinarily be more than £750.

Applications should be made on forms to be obtained from the Executive Secretary, The Royal Society, 6 Carlton House Terrace, London SW1Y 5AC, and returned by December 15, 1978. 8136(A)

COURSES

EMBO LABORATORY COURSE

ON

"Plasmids—A Course in Advanced Techniques"

April 9-29, 1979

UNIVERSITY OF
ERLANGEN, GERMANYOrganisers: Kenneth Timmis and
Alf Pühler

The course will consist of laboratory experiments and lectures designed to instruct participants in the use of recently developed methods that are particularly important for the structural and functional analysis of plasmid genomes. These will include transposition mutagenesis, gene cloning, the uses of restriction enzymes, electron microscopy and DNA sequencing for structural analysis, the uses of minicells and *in vivo* systems for studying plasmid expression and replication.

A mini-symposium on the "Plasmids of Medical, Environmental and Commercial Importance" will follow the laboratory course.

Applications, with a short curriculum vitae including future scientific interests, and any information which might help in their evaluation should be sent in duplicate to:

Professor A. Pühler
Institute of Microbiology and
Biochemistry
University of Erlangen
Friedrichstr. 33
D 8520 Erlangen, FRG 8109(I)

NATO ASI

NUCLEOSIDE ANALOGUES: CHEMISTRY, BIOLOGY AND MEDICAL APPLICATIONS

May 7-18, 1979

Sogesta, nr. Urbino, Italy

This interdisciplinary Study Institute will be directed towards the *Structural synthesis and chemical reaction nucleosides, their evaluation and as antiviral and anticancer drugs.* The programme will include review lectures, research seminars and discussion periods.

The lecturers will include: J. van Boom (Netherlands), Y.-C. C. (USA), S. S. Cohen (USA), S. Danyluk (USA), T. C. Merigan (U.S.), J. G. Moffatt (USA), W. H. P. (USA), M. J. Robins (Canada), Saenger (Germany), F. M. S. (USA), R. W. Sidwell (USA), L. Townsend (USA), H. Vorbrüggen (Germany).

Applications should reach Dr R. Walker, Department of Chemistry, Birmingham University, P.O. Box, Birmingham B15 2TT, UK, at or not later than December 1, 1978.

They should contain a short background and interests and a letter of recommendation where appropriate. Research seminars to be presented by applicants. A short abstract of contributions should accompany application. The number of participants will be limited to 60 and everyone is expected to attend for the duration of the course.

Successful applicants will have to provide a non-refundable deposit of US\$100 (which will then be deducted from the living expenses of ~\$390) limited number of stipends, to cover partially the living expenses and travel costs are available but participants are encouraged to cover their expenses from the resources of their institutions wherever possible. 8158(I)

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